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ERNEST ADAM DOPP
1896–1944

R. D. Rands

The untimely death on September 24, 1944, of Mr. Ernest A. Dopp cut short a productive investigator in the prime of his career. Mr. Dopp was Assistant Pathologist in charge of the U. S. Sugar Plant Field Station at Canal Point, Florida. There he collaborated on sugar-cane breeding and the testing of varieties for disease resistance.

He was born September 12, 1896, at Superior, Wisconsin, the younger son of a family of horticulturists and gardeners. He graduated in 1917 from a teachers' training course at the Superior Normal School and was immediately drawn into the military service of World War I. Upon discharge, he taught for a year in high school and in 1920 entered the University of Wisconsin. There he obtained the B.A. degree in botany in 1922 and M.A. in botany and plant pathology in 1924. From 1924 to 1927 he was an instructor in botany at the University of Minnesota where he pursued some further graduate studies.

Dopp's service in the Bureau of Plant Industry, U. S. Department of Agriculture, extended from September, 1928, until his death; first, for two years in collaboration with the late W. W. Gilbert on joint investigations of cantaloupe and melon diseases, and from 1930 to 1940 with the present writer on diseases of sugar cane. He became an authority on identification of species of *Pythium* connected with root rots and made extensive studies of the mosaic virus and fungus leaf diseases, most of which remain unpublished. In 1940, he was transferred from Washington, D. C., to Canal Point, Florida, to take charge of the Department's sugar-cane breeding station where his cane disease studies along with varietal resistance tests were continued.

Dopp had a special gift in mathematics, which led him into extensive studies of statistical methods in agriculture and especially experimental designs for greenhouse and field tests. Because of his reluctance and even dislike of formal publication, this work might have been lost but for his unselfishness in communicating his ideas and results to associates.

Ernest Dopp exemplified to the highest degree those essential qualifications of modesty, generosity, an analytical mind, adept craftsmanship, and technical ability for effective teamwork and harmonious cooperative research. Thus, in the later years he became an indispensable member of the sizable group of federal and state workers devoted to the closely coordinated sugar-cane improvement projects. Although results of work in which he was engaged are unsuited for frequent individual publication they have been none the less spectacular in the constantly increased yields of sugar cane throughout the Gulf States. His loss will be sorely felt.

ERNEST ADAM DOPP
1896–1944

He was author of the following publications:

An epidemic outbreak of red stripe disease of sugar cane and the reaction of some seedling progenies. 4th Cong. Internatl. Soc. Sugar Cane Technol. Proc. Bull. 46. 1932. (With R. D. RANDS.)

Variability in *Pythium arrhenomanes* in relation to root rot of sugar cane and corn. Jour. Agr. Res. [U.S.] **49**: 189–221. 1934. (With R. D. RANDS.)

Influence of certain harmful soil constituents on severity of Pythium root rot of sugar cane. Jour. Agr. Res. [U.S.] **56**: 53–67. 1938. (With R. D. RANDS.)

Pythium root rot of sugar cane. U. S. Dept. Agr. Tech. Bul. 666. 1938. (With R. D. RANDS.)

VIABILITY AND INFECTION OF LIGHT AND HEAVY COTTON SEEDS[1]

C. H. Arndt

(Accepted for publication April 20, 1945)

Chester[2] has published observations on the germination of acid-delinted cotton seed which had been separated into light and heavy seeds on the basis of their specific gravity relative to that of water. His generalized data indicate superior value for the heavy seeds. Similar methods have been used to separate sound from defective seeds in other plant species.[3] This paper is a summary of studies made or supervised by the writer to ascertain the general applicability of Chester's observations to typical lots of cotton seed produced in the United States.

METHODS

The seeds were prepared by a method similar to that used by Chester. About 300 g. of seeds of each lot were acid-delinted with concentrated sulphuric acid, after which the acid was removed by washing with water. During this process, but when not agitated, the seeds were separated into floating and submerged, or light and heavy, seeds. The two fractions were then air-dried and stored in the laboratory until it was convenient to test germination. The percentages of light and heavy seeds were ascertained from their air-dry weights.

Several methods were used to determine the percentage of germination. The 1934 lots of seed were germinated on paper toweling in covered trays at 30° C. In later studies, Ceresan-treated seeds were germinated in trays of steamed sand. For each test, 100 seeds regularly spaced and planted at a uniform depth were observed for 14 days or slightly longer.

When it was desired to ascertain the percentages of infected seeds, from 40 to 80 seeds of each sample of light and heavy seeds were germinated on sterile non-nutrient agar in test tubes, one seed to each tube. Immediately before germination, the seeds were surface-sterilized by immersion for 2 minutes in a solution of $HgCl_2$ in 50 per cent ethanol (2.5 g./1.), after which they were washed briefly with sterile water. Previous studies had indicated that Ceresan, which is commonly used to treat seed before planting in the field, could not be used, because of its adverse effect on germination when seeds are germinated on agar. The seeds were incubated for 14 days at 23° C. At the end of this period, notes were taken on germination; and the infecting fungi, when present, were identified. All data have been adjusted to a percentage basis for convenience in making comparisons.

[1] Technical contribution No. 123 of the South Carolina Agricultural Experiment Station. Investigation made in cooperation with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Chester, K. S. Gravity-grading, a method for reducing seed-borne disease in cotton. Phytopath. 28: 745–749. 1938.

[3] Baldwin, H. I. Alcohol separation of empty seed, and its effect on the germination of red spruce. Amer. Jour. Bot. 19: 1–11. 1932.

RESULTS

Relative Percentages and Weights of Light and Heavy Seeds. The results, as summarized in tables 1, 2, 3, and 4, show a range in the percentage of light seeds from 4 for the variety Half and Half to 94 for the variety Qualla (both in table 1).

The percentage of light seeds was relatively high in all lots of the variety Wilds, the lowest percentage being 47 (Table 1). The percentages for 9

TABLE 1.—*Percentage germination, weight per 100 seeds, and percentage of light seeds for 18 lots of cotton seed of the crop of 1934, arranged in order of percentage of light seeds*

Variety	State of origin	Weight per 100 seeds	Germination	Light seeds
		Grams	*Per cent*	*Per cent*
Half & Half	Ga.	8.6	94	4
Cook	Ala.	9.0	86	5
Dixie Triumph	S. C.	9.8	61	13
Stoneville	Miss.	8.7	91	16
Mexican Big Boll	N. C.	12.5	93	16
DPL–11	Miss.	8.7	82	19
Dixie Triumph	S. C.	9.4	74	21
Cleveland	S. C.	9.3	58	23
Triumph	Okla.	10.5	65	24
Arkansas No. 17	Ark.	10.6	95	31
Super–7	S. C.	10.7	70	33
Missdel	Miss.	9.7	78	36
Farm Relief	S. C.	11.8	69	38
Wilds	S. C.	12.5	80	47
Startex	Texas	10.0	89	59
Rowden	Ark.	10.8	90	63
Acala	Okla.	11.0	89	69
Qualla	Texas	10.0	86	94

other lots of Wilds seed, representing the 1938 to 1941 crops, were 70, 80, 60, 94, 93, 84, 94, 80, and 63. The last 6 lots listed were grown in 1940 in different localities of northeastern South Carolina. The germination of the light seeds of this variety was generally high, or 75, 85, 90, 3, 88, 79, 69, 42, and 77 per cent, respectively, for the nine lots. The corresponding percentages for the heavy seeds were 90, 92, 92, 22, 80, 91, 84, 80, and 95. The low viability of the fourth lot was due to exposure to prolonged rainfall in the field before picking. The mean seed weight of the variety Wilds was generally high, as shown by the mean weights of 12.5 g. and 11.5 g. per 100 nongraded seeds (Tables 1 and 2).

That high seed weights tend to be associated with high percentages of light seeds in other varieties is indicated by the data of the several tables. Thus, if the lots listed in tables 1, 2, 3, and 4 are arranged into groups according to the mean weights of the seed, or weights of 8.0 to 8.9, 9.0 to 9.9, 10.0 to 10.9, and 11.0 to 12.5 g. per 100 seeds before flotation grading, the mean percentages of light seeds for each group will be 17, 27, 36, and 41, respectively. Similarly, although grown in the same county and presum-

TABLE 2.—*Comparative data on sinkers and floaters in 12 lots of cotton seed produced in 1938*

Variety and State of origin	Light seeds	Weight of 100 seeds		Germination		Light seeds after vacuum[a]
		Heavy seeds	Light seeds	Heavy seeds	Light seeds	
	Per cent	*Grams*	*Grams*	*Per cent*	*Per cent*	*Per cent*
Mexican B.B., N. C.	6	11.6	12.4	91	82	58
Farm Relief, S. C.	19	9.3	8.5	82	80	.....
Carolinadel, S. C.	21	9.8	8.2	90	65	.....
Acala, Calif.	22	12.3	11.7	89	82	.....
Dixie-Triumph, S. C.	27	10.1	9.6	93	68	.....
DPL, S. C.	27	9.7	8.4	96	77	.....
Miller, Miss.	27	10.6	9.5	91	69	.....
DPL, S. C.	33	10.2	8.1	72	71	30
Dixie-Triumph, S. C.	38	10.0	9.8	83	75	12
Farm Relief, S. C.	42	10.0	9.6	96	84	12
Wilds, S. C.	70	12.6	11.0	90	68	28
Acala, Okla.	81	7.9	7.0	34	14	11

[a] Percentage of light seeds of original separation which still floated after being subjected to a vacuum equivalent to 50 cm. of mercury while immersed in water.

ably from the same original seed stocks, the four lots of Coker 4-in-1 (Table 3) have mean weights per 100 nongraded seeds of 9.3, 9.9, 10.0, and 10.4 g., and the percentages of floaters are 10, 19, 23, and 50. Notable exceptions to the tendency of high seed weights to be associated with a high percentage of light seeds are shown by the Mexican Big Boll lots of high seed weight (Tables 1 and 2) and the Acala lot of low seed weight (Table 2).

TABLE 3.—*Comparative data on heavy and light seeds in 20 lots of cotton seed produced in South Carolina in 1939*

Variety	Light seeds	Weight of 100 seeds		Germination		Weight of 100 embryos	
		Heavy seeds	Light seeds	Heavy seeds	Light seeds	Heavy seeds	Light seeds
	Per cent	*Grams*	*Grams*	*Per cent*	*Per cent*	*Grams*	*Grams*
Coker 4-in-1	10	9.5	7.3	88	73	.....	.....
Marett–100	11	10.2	6.5	83	43	6.70	4.22
Stoneville	11	10.7	7.6	64	41	.....	.....
Stoneville	12	11.1	8.6	67	79	.....	.....
Coker–100	16	9.5	7.4	69	28	.....	.....
Coker 4-in-1	19	10.3	8.1	80	57	.....	.....
DPL	19	9.1	7.3	65	26	.....	.....
White Gold	21	11.1	8.6	89	67	6.65	5.25
Dixie-T	23	9.6	7.4	87	84	.....	.....
Coker 4-in-1	23	10.6	8.0	86	71	.....	.....
Coker–100	28	9.9	7.4	87	44	.....	.....
Dixie-T	31	9.9	8.3	60	24	6.61	5.68
Stoneville	38	10.7	8.4	70	47	.....	.....
Clevewilt	38	10.3	8.9	74	45	6.33	4.68
Wonderwilt	39	11.1	8.8	82	46	.....	.....
Dixie-T	40	10.7	9.9	68	33	6.56	6.42
Maretts-WR	44	10.6	10.4	80	38	7.12	6.26
Dixie-T	47	9.4	9.1	52	23	6.18	5.73
Clevewilt	48	10.4	9.3	51	7	.....	.....
Coker 4-in-1	50	10.7	9.4	54	35	.....	.....

The weight of the heavy seeds was greater than that of the light seeds for all of the 37 lots listed in tables 2, 3, and 4, except for the first lot in table 2 and the Coker-100 lot of 1943 in table 4. The greatest difference between the weights of the heavy seeds and light seeds was that for the second lot in table 3, the weight of the latter seeds being only 63 per cent of that of the former. For 10 other lots (Table 3) the weight of the heavy seeds was at least 20 per cent greater than that of the light seeds.

In order to ascertain whether the low specific gravity of the light seeds might be partly due to occluded air, light seeds of six of the 1938 lots (Table 2) were subjected, while immersed in water, to a vacuum equivalent to approximately 50 cm. of mercury for three 2-minute periods, about 3 minutes elapsing between the successive vacuum treatments. After this treatment only 11 to 58 per cent of the original light seed still had a specific gravity less than that of water. This suggests that the difference between light and heavy seeds, in part, may be due to the greater amount of air occluded in the light seeds.

TABLE 4.—*Comparative data on sinkers and floaters of acid-delinted seed used in field plantings in 1941 and 1943. Seed produced the preceding season*

Year, variety, kind of seed, and State of origin	Light seed	Weight per 100 seed	Laboratory tests	
			Viable seed	No. seedlings diseased
	Per cent	*Grams*	*Per cent*	
1941 Plantings[a]				
Coker–100, S. C.	21			
Heavy seed		9.2	91	0
Light seed		8.3	87	10
DPL–12a, Miss.	27			
Heavy seed		8.54	85	0
Light seed		7.11	81	1
Acala, Okla.	55			
Heavy seed			80	0
Light seed			80	0
1943 Plantings				
Coker–100, S. C.	66			
Heavy seed		11.74	99	18
Light seed		11.84	89	22
Delfos–651, Miss.	8			
Heavy seed		9.47	94	9
Light seed		8.61	66	9

[a] These lots of seed were used in 19 plantings sponsored by the plant pathologists of the Southern States to evaluate the grading of acid-delinted seed. Detailed data will be published later by the supervising committee.

Since a small percentage of the light seeds did not contain embryos, it was thought likely that some of them might contain immature embryos and, consequently, that the proportion of the total seed weight represented by the weight of the embryos might be smaller in the light than in the heavy seeds. Relative weights of the embryos of heavy and light seeds were ascertained for 7 lots (Table 3). The embryos of these same lots of seed ranged from 60 to 67 per cent of the total weight of the seed for the heavy seeds and

from 52 to 68 per cent for the light seeds, with no clear tendency for the proportional weight to be greater for either class; but, for each lot the weight of 100 embryos of the light seeds was less than that for the embryos of the heavy seeds.

To ascertain the influence of the crop year on the relative proportions of light and heavy seeds, 14 lots of seed, representing 8 varieties, were collected in 1940 and 1941 from the same 4 counties of South Carolina—one county being selected as representative of each of the following sections of the State: northeastern, south-central, north-central, and northwestern. The percentage of light seeds for all lots of the same variety from the same locality was greater in 1941 than in 1940. A lot of the variety Wilds had the highest percentage of light seeds in both 1940 and 1941, or 64 and 85, respectively. For the other 13 lots, the respective mean percentages for the 1940 and 1941 lots were 9.7 and 14.3. For these same 13 lots in 1940, the lowest and highest percentages of light seeds were 5.3 and 15, respectively; and for 1941, 10.3 and 22, respectively; while the greatest difference between the percentage of light seeds in these two years for any one of these varieties from the same county was 8 per cent. Thus, although these data show that there may be variation in the precentage of light seeds from year to year for a given variety, the varieties tend to maintain the same relative order in a classification based on the percentage of light seeds.

One-half of each of the 14 lots of 1940 seed was delinted in September, 1940; and the remaining half was similarly delinted a year later, after storage in paper bags in the laboratory. The percentages of light seeds at both times were almost identical; thus, storage had no definite effect on the proportions of heavy and light seeds.

Germination of Heavy and Light Seeds. The germination of the heavy seeds for the 37 lots (Tables 2, 3, and 4) was equal to or greater than that of the light seeds, except for what might be a questionable instance for the fourth lot in table 3. Generally, the difference in germination between the two fractions was least when the germination of heavy seeds was relatively high. The lowest germination of light seeds relative to that of the heavy ones was 13 per cent (Lot 19, Table 3). For all 37 lots the mean germination of the heavy seeds was 62.5 per cent; that of the light seeds, 44 per cent.

Infection of Heavy and Light Seeds. Chester suggested that the removal of the light seeds might greatly reduce the incidence of disease in a cotton planting, since these seeds might be expected to be more highly infected by potential pathogens than the heavy seeds. For information on the relative infection, seeds of the two fractions of the 37 lots were germinated to ascertain the percentages of infected seeds and, when possible, the infecting fungi. As the differences in infection between the two fractions were about the same for all lots, the relevant data on infection have been summarized in table 5 without reference to the individual lots.

The heavy seeds were by no means necessarily free of pathogenic fungi, although the number infected was slightly less than in the case of the light seeds, except for the numbers infected by *Fusarium spp.*

The difference between them was small for *Colletotrichum gossypii* South., the most important of these infecting fungi. This fungus was found largely on seedlings and partially germinated seeds, as indicated in table 5, and was obtained in only a relatively few cases from the nongerminating seeds. In the latter, the presence of this fungus may have been obscured by other more rapidly growing fungi; for in the examination of nongerminating seeds, the first fungus to be identified was recorded as the fungus present, unless mycelial characteristics indicated the possible presence of other fungi. Thus, only one fungus was generally listed for a given seed, although the presence of other fungi cannot be excluded.

TABLE 5.—*Relative infection of heavy and light seeds*

Kind of seed	Total No. seeds infected	Number of seeds and seedlings infected by various fungi or bacteria							
		Colletotrichum gossypii	*Fusarium moniliforme*	*Fusarium spp.*	*Rhizopus sp.*	*Penicillium spp.*	*Diplodia theobromae*	Miscellaneous fungi	Bacteria
Data for 1220 seeds of both fractions of the 37 lots in tables 2, 3, and 4									
Heavy	141	53	17	30	0	1	27	10	8
Light	264	69	32	29	3	8	70	33	12
Data for 500 seeds of both fractions of the 5 lots in table 4									
Normal seedlings									
Heavy	20	6	10	1	0	0	2	0	1
Light	19	3	13	2	0	0	0	1	0
Partial germination (radicals formed but no cotyledons)									
Heavy	5	3	3	0	0	0	0	0	0
Light	25	9	7	1	3	0	2	3	0
Nongerminating seeds									
Heavy	7	1	0	2	0	0	0	0	4
Light	41	2	5	7	3	8	8	5	5

Fusarium moniliforme Sheld. (Table 5) also occasionally caused lesions, ranging from small to extensively decayed areas on the cotyledons of seedlings. This fungus and *Colletotrichum gossypii* infected a number of the partially germinated seeds. The failure to form normal seedlings was due, at least in part, to infection by these two fungi. *F. moniliforme* appeared more frequently on nongerminating seeds than *C. gossypii*. Other Fusarium species, largely of the Gibbosum section, were occasionally found on seedlings, but more frequently on the nongerminating seeds, as were also such fungi as, *Rhizopus spp.*, *Penicillium spp.*, *Diplodia theobromae* (Pat.) Nowell (*D. gossypina* Cke.), miscellaneous fungi (in part *Alternaria spp.*, *Aspergillus spp.*, and fungi not forming spores while under observation), and bacteria. *D. theobromae* appeared more frequently on the nongerminating light seeds than on those of the heavy seeds.

Upon the suggestion of Dr. W. W. Ray that it might be possible to separate viable from non-viable seeds by appropriate water-ethyl alcohol mixtures, seeds of 5 lots of the variety Wilds (1940 crop) were flotation-graded in such alcohol solutions. There was a steady fall in the percentage of floating seeds for the five lots from 93, 94, 94, 80, and 73, respectively, in water; to 22, 43, 64, 43, 36, respectively, in 50 per cent alcohol; and to 8, 12, 25, 19, and 19, respectively, in 80 per cent alcohol. The percentage of alcohol necessary to obtain floating seeds of appreciably lower viability than those obtained in water ranged from 20 to 60. The mean germination of the seeds of these five lots which floated in water was 71 per cent; that of those which floated in 60 per cent alcohol, 54 per cent. Thus, although it may be possible to make a partial separation of viable and non-viable seeds by such solutions, this method appears to have no practical application.

SUMMARY

Seeds of lots of the upland varieties of cotton, *Gossypium hirsutum* L., were acid-delinted and then separated into two fractions, light and heavy seeds, on the basis of their specific gravity relative to that of water. After separation the fractions were dried, relative mean weights ascertained, and seeds of each germinated to ascertain their viability and infection by fungi and bacteria.

The light seeds in these lots ranged from 4 to 94 per cent, and the relative proportions of light and heavy seeds were determined more largely by varietal characteristics than by the viability of the seed, internal infection by fungi, or crop year. Varieties with high seed weights tended to have the highest precentages of light seeds. The weights of the embryos of the light seeds were less than those for the heavy seeds. The relative proportion of the total seed weight represented by the embryo was about the same for both. Removal of the air between the seed coat and embryo and the infiltration of this space with water changed most of the floating to submerged seeds.

The light seeds were somewhat more highly infected internally by fungi than the heavy seeds. The differences between the two in infection by the important seedling pathogens, *Colletotrichum gossypii* and *Fusarium spp.*, were relatively small. The differences in infection were relatively large for such fungi as *Penicillium spp.*, *Diplodia theobromae*, and *Aspergillus spp.*

The viability of the light seeds was generally less than that of the heavy seeds when the percentage of light seeds was small; but, when the percentage of the latter equalled or exceeded that of the heavy seeds, the viability of the light seeds approached that of the heavy seeds.

Improvement in seed quality which may be attained by water grading is determined by the characteristics of each lot of seed, and its general applicability for the improvement of seed quality is questionable.

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VARIETAL VARIATION AND INHERITANCE STUDIES ON NATURAL WATER-SOAKING IN TOBACCO[1]

HOWARD E. HEGGESTAD

(Accepted for publication April 28, 1945)

The predisposing effect of natural water-soaking[2] in leaf tissues to infection and development of tobacco wildfire [*Phytomonas tabaci* (Wolf and Foster) Bergey *et al.*] and blackfire [*Phytomonas angulata* (Fromme and Murray) Bergey *et al.*] has been shown recently. Some variation in resistance had been reported previously in varieties of tobacco (*Nicotiana tabacum* L.) to these bacterial leaf-spot diseases, but varietal behavior under experimental trials had not been consistent, apparently because of wide differences in conditions provided for infection. Under field conditions, disease development may occur as a result of either storm water-soaking (7) or natural (internal or physiological) water-soaking (14). Varietal behavior toward disease may be expected to be different under these circumstances. One of the purposes of this study is to show that varietal variation in susceptibility to natural water-soaking occurs in tobacco, and that this is correlated with variation in susceptibility to the wildfire disease. Special efforts were made to study the inheritance of natural water-soaking after distinct varietal differences were found. The data presented are believed to be sufficient to show that the character of natural water-soaking is inherited in much the same way as many other quantitative plant characters, and that this character is closely associated with susceptibility to the wildfire disease of tobacco.

LITERATURE REVIEW

As far as is known, no previous work has been done on the varietal behavior and the inheritance of natural water-soaking in plants. The author has, however, recently presented (13) summarized results of some of the investigations reported in this paper.

Johnson (14) in 1936 first discussed natural water-soaking and its probable relation to infection and development of epidemics of blackfire and wildfire of tobacco. The following year, he (15) reported on further results with the artificial internal water-soaking method; that is, water-soaking by means of applying high water-pressure directly to cut stems or exposed roots. Many species water-soaked in this manner became infected easily when inoculated with weak pathogens such as the tobacco blackfire organism. Previous attempts to secure good infection with blackfire bacteria without water-soaking had failed even on tobacco. A close correlation was observed by Braun

[1] This paper was presented to the Graduate School of the University of Wisconsin in partial fulfillment of the requirement for the degree of Doctor of Philosophy. The writer is greatly indebted to Dr. James Johnson for advice and criticism during the work and the preparation of the manuscript.

[2] "Natural water-soaking" is synonymous with the more descriptive term "water-congestion" suggested in a recent publication by Johnson (16).

and Johnson (5) between the amount of natural water-soaking found in farmers' plant beds and subsequent amounts of blackfire developing in the same plant beds. Johnson *et al.* (18) more recently reported, on the basis of observation, that natural water-soaking was apparently a genetically variable phenomenon and was therefore concerned with susceptibility to disease. It was on the basis of this observation that the present investigations were undertaken.

Proof of the bacterial origin of tobacco wildfire and tobacco blackfire was not established until 1918 and 1919. In the following decade, several investigators (3, 6, 8, 11) noted differences in varietal resistance to these leaf-spot diseases, but for the most part they were inconsistent or were regarded as of minor significance hardly worthy of consideration as a control measure in improving commercial varieties of tobacco. More recently, Garner *et al.* (12) reported that a selection of the Narrow-Leaf Orinoco variety grown in Virginia showed some resistance to blackfire. Johnson and Ogden (17) found the variety Havana 211 to be more susceptible to blackfire and wildfire than other local varieties following epidemics of the disease in the field; and Valleau *et al.* (19) reported Dark tobacco to be more resistant to angular leaf spot (blackfire) than Burley tobacco grown in the same plant bed. Hence, there is considerable observational evidence of varietal differences in resistance to bacterial diseases of tobacco. There is, however, a lack of consistent experimental evidence, and the reasons for this are more obvious now since there are two very distinct means of infection by the pathogens; namely, through "storm water-soaking" and "natural water-soaking."

METHODS AND MATERIALS

Methods. Tobacco seedlings that had been grown in steamed compost soil were transplanted, before the largest leaves were 20 mm. across, to 9 by 14-inch flats of steamed, low potash (2), sandy loam soil. About 2 dozen flats, each commonly planted with 10 plants of 3 different tobacco strains, were in each series. Two weeks after transplanting the tobacco had grown until the soil was almost covered; the seedlings were then ready for treatment. Extensive natural water-soaking in the most susceptible strains was induced by 24 to 48 hours in the moist chamber, from which the plants quickly recovered when returned to the greenhouse bench. One treatment each week for 3 consecutive weeks was usually made with the tobacco.

The moist chamber, located in a dark (1, 18) basement, had a fairly constant temperature that varied within only a few degrees of 65° F. during the entire greenhouse season. The relative humidity was maintained at 100 per cent by saturating the atmosphere with a fine mist from atomizers connected to an air line and kept in continuous operation. After sufficient exposure in the chamber, one flat of seedlings at a time was removed to a lighted room and the amount of water-soaking determined by examination of each plant.

A scoring system, comprised of 9 classes ranging from 0 to 4, was selected

as the most convenient method of recording the degree of water-soaking. The 4 major divisions or classes (scored 1, 2, 3, and 4) are illustrated in figure 1. Intermediate classes (scored 0.5, 1.5, 2.5, and 3.5) were also used, and plants with no macroscopically visible water-soaked areas on any of the leaves were scored 0. The 4 major classes may be described as follows:

Class 1. Water-soaking at the tip of one or more leaves, but largely confined to the leaf margin. The most heavily water-soaked leaf on the plant showing not more than an estimated 5 per cent of its leaf area affected.

Class 2. Water-soaking on one or more leaves, interspersed about most of the leaf margin and small amounts on other parts of the leaf blade. Approximately 5 to 20 per cent of the leaf area affected.

Class 3. Water-soaking as described for Class 2, except more of the leaf area (20 to 35 per cent) water-soaked.

Class 4. The most heavily water-soaked leaf, having numerous scattered water-soaked areas and an estimated 35 per cent or more of the leaf area water-soaked. Occasionally as much as 80 per cent of the leaf area was water-soaked under the conditions of the experiment. Leaves in classes 3 and 4 occurred infrequently under average experimental conditions.

TABLE 1.—*Representative data showing arbitrary system of estimating natural water-soaking (score 0–4) on individual plants at three different times of exposure in moist chamber*

Parent or F_3 family	Consecutive exposures in moist chamber	Plant and estimated water-soaking score					
		1	2	3	4	5	Ave.
American (susceptible)	First	2.5	3.0	2.5	3.0	2.5	2.7
	One week later	2.5	2.0	2.0	2.5	2.5	2.3
	Two weeks later	1.5	0.5	1.0	0.5	1.0	0.9
Daruma (resistant)	First	0.0	0.0	1.0	0.5	0.0	0.3
	One week later	0.0	0.0	0.0	0.0	0.5	0.1
	Two weeks later	0.0	0.0	0.0	0.0	0.0	0.0
American × Daruma F_3 44	First	1.5	1.5	2.5	3.0	2.5	2.2
	One week later	1.5	2.0	1.5	1.5	1.0	1.5
	Two weeks later	1.0	0.5	1.0	1.0	1.5	1.0
American × Daruma F_3 45	First	0.0	0.0	2.0	1.5	2.0	1.0
	One week later	0.0	0.0	1.5	0.5	0.0	0.4
	Two weeks later	0.0	0.0	1.0	0.0	0.0	0.2

The detailed method of scoring is illustrated in table 1, showing representative data on 5 plants of each of 2 varieties and 2 F_3 families, together with the results of 3 consecutive exposures of the same series. The amount of water-soaking decreased with repeated exposures, but the same relative proportions were maintained between susceptible and resistant varieties or families. The figures presented in the other tables as water-soaking score or percentage of plants water-soaked represent the average behavior of the material in all exposures in the moist chamber.

FIG. 1. Tobacco leaves illustrating arbitrary classes of water-soaking, as used in recording results. Numbers refer to classes of water-soaking.

Materials. All important local varieties and strains and representative strains of the more important domestic varieties of tobacco (*Nicotiana tabacum* L.) have been tested. In addition, 103 varieties and strains of tobacco, mostly South American, were secured through the cooperation of Dr. E. E. Clayton who had arranged for an extensive collection of tobacco seed by the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C. Limited trials were made with 13 other species of *Nicotiana.* Several varieties of tomato (*Lycopersicum esculentum* Mill.), oats (*Avena sativa* L.), and corn (*Zea mays* L.) were kindly supplied by associates at the Wisconsin Experiment Station for other phases of the investigations.

EXPERIMENTAL RESULTS

Other workers (2, 18) have demonstrated that natural water-soaking in plants is influenced by the nature of the soil in which the plants are grown. Soils low in available potash favor natural water-soaking. In preliminary work on selection of a suitable soil, 50 small lots of soils from the sandy northwestern section of Dane County, Wisconsin, were compared as to their ability to influence water-soaking in tobacco plants. The maximum water-soaking (average score of 2.83) occurred with the sandy loam sample No. 23, and none appeared in plants grown in compost commonly used for other types of greenhouse experiments. In three soil samples, the sandy-loam K, silt-loam G, and the greenhouse compost, the available potash an acre amounted to 177, 715, and 3,030 pounds, respectively; and the average water-soaking scores for plants grown in these respective soils were 2.42, 1.92, and 0. Sandy-loam K was selected for most of the experimental work in the greenhouse. The soil was steamed in flats at 100° C. for 30 minutes to destroy damping-off organisms and weed seeds. The steaming also liberated some nitrogen and thus augmented the water-soaking in plants subsequently grown in the soil.

Older seedlings, in the greenhouse and in outdoor plant beds, are not water-soaked as easily as younger seedlings. Experimental plants were tested for water-soaking 3 times (Table 1) at weekly intervals beginning about two weeks after transplanting. In one comparison, involving 1080 plants, 91, 60, and 25 per cent of the plants water-soaked at 2, 3, and 4 weeks respectively after transplanting.

In outdoor tobacco plant beds under cloth covers, plants of all varieties may water-soak as soon as the first two leaves are unfolded and may continue to remain highly susceptible until the largest leaves are one inch or more in length. Some of the varieties classed as fairly resistant are rather susceptible as very young plants. However, as they become older, resistant varieties are rarely water-soaked under favorable weather conditions. Examination by Johnson and others (5, 18) have shown typical water-soaked areas on a variety of plants in various stages of maturity. For purposes of our investigation, it seemed desirable to limit the work and conclusions largely to young seedling plants.

Varietal variation in Nicotiana tabacum

The species *Nicotiana tabacum* contains hundreds of varieties or strains that are grown commercially in various parts of the world. Approximately 150 varieties and strains, including both domestic and foreign varieties, were grown in the seedling stage and compared for behavior in water-soaking. Out of these trials, 7 varieties were selected for further study as representing both extremes and intermediates in behavior. These were later compared in more critical tests as shown by summarized data from two experiments presented in table 2. The results of the two separate trials are in fairly close

TABLE 2.—*The relative score and percentage of plants water-soaked in seven varieties and crosses between these varieties in the F_1 and F_2 generation*

Designation	Experiment 1[a]		Experiment 2[b]	
	Score	Plants water-soaked	Score	Plants water-soaked
	Average	*Per cent*	*Average*	*Per cent*
Parents				
American (susceptible)	2.36	100	1.69	88
Vastanhog	1.74	100	1.54	92
Wisconsin Seedleaf	1.40	80	0.64	45
Samsun	1.06	63	0.25	35
Havana 211	0.23	23	0.36	40
Havana 142	0.10	13	0.20	25
Daruma (resistant)	0.09	8	0.21	25
Crosses	F_1 generation		F_2 generation	
American × Vastanhog	1.88	100	1.48	94
American × Wisconsin Seedleaf	1.53	100	1.58	91
American × Samsun	1.05	68	1.14	69
American × Havana 211	0.63	60	0.96	80
Vastanhog × Samsun	0.56	48	0.91	76
American × Daruma	0.41	58	0.92	73
Vastanhog × Daruma	0.79	68	0.72	70
Wisconsin Seedleaf × Daruma	.....	.....	0.72	66
Wisconsin Seedleaf × Havana 142	0.29	30	0.46	43
Samsun × Daruma	0.00	0	0.51	41
Havana 211 × Daruma	0.03	5	0.38	45
Havana 211 × Havana 142	0.00	0	0.18	26

[a] Two replications of ten plants each.
[b] Parents three replications, and F_2 progeny four replications of ten plants each.

agreement and seem to justify the belief that similar trials could be conducted throughout the greenhouse season with comparable results.

The two foreign varieties, American and Daruma (Fig. 2), represent extremes in susceptibility and resistance in greenhouse tests. In other experiments under conditions less favorable for water-soaking or when exposed for only a few hours to conditions favoring water-soaking, the variety American was not as extensively water-soaked as shown in figure 2, but the variety Daruma then failed to show any signs of water-soaking.

Similar differences in varietal susceptibility were also found in limited tests with half-grown plants of the varieties American, Wisconsin Seedleaf, and Daruma. Plants were grown in "lake sand" in 2-gallon glazed crocks

and supplied with modified (without potash) Hoagland's nutrient solution. Symptoms of potash deficiency were evident by the time the plants were transferred to the moist chamber. No marked differences were found in the degree of water-soaking between the two susceptible varieties American and Wisconsin Seedleaf which were both extensively water-soaked, but the resistant variety Daruma showed only slight water-soaking on 1 out of 12 plants tested. It is significant that the relatively large plants of these varieties behaved in the same manner as small seedlings in other tests.

In 1936 Dr. E. E. Clayton, of the Bureau of Plant Industry, found (correspondence) that the variety American was one of the most susceptible and

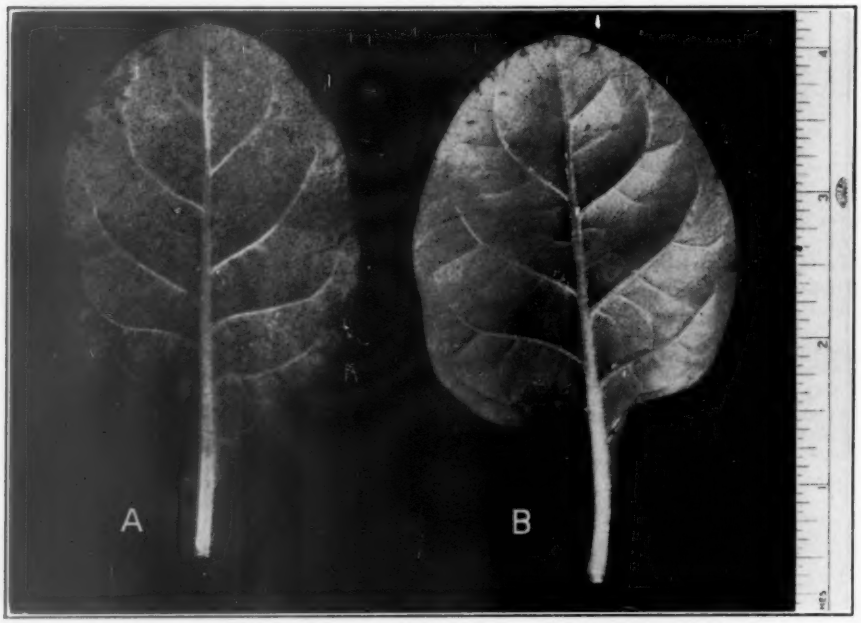

FIG. 2. Water-soaking of tobacco leaves showing extreme differences that may be secured under identical environmental conditions. A. American. B. Daruma.

the variety Daruma was the most resistant to storm water-soaking among approximately 100 varieties and strains of *Nicotiana tabacum* tested. The tests were made on nearly mature field-grown plants in connection with studies on resistance to disease (7, 12). The factors involved up to a certain stage may conceivably be similar to those concerned with natural water-soaking.

Species variation in Nicotiana

Fourteen species of *Nicotiana*, tested under moist-chamber conditions, varied in susceptibility to natural water-soaking from those very resistant but not immune to those which were nearly as susceptible as *N. tabacum*. The water-soaking pattern (that is, the size and distribution of water-soaked areas in the leaf) differed on some of these species. Consequently, it was

more difficult to make comparisons between these species than between the varieties of *N. tabacum*. For example, water-soaked plants of *N. paniculata* L. and *N. glutinosa* L. developed numerous small (many microscopic) water-soaked areas that were rather evenly distributed about the leaf. Water-soaked plants of *N. tabacum* L. and *N. rustica* L. developed, however, relatively large and conspicuous water-soaked areas that appeared first along the margin of the leaves. *N. nudicaulis* Wats., *N. repanda* Willd., and *N. rustica* L. were the most resistant of the species in our trials.

Inheritance of natural water-soaking

The F_1 and F_2 generation. The F_1 progeny from 11 crosses are compared in an experiment with an equal number of plants of the parents in table 2. The figures presented are the average calculations of two exposures in the moist chamber for 24 hours each, with a one-week interval between trials. For more ready comparison, the parents and progeny are arranged in the order of increasing resistance.

In the cross American (susceptible) and Daruma (very resistant), the F_1 generation was intermediate in susceptibility to water-soaking. The F_1 of the cross between the two most susceptible varieties, American and Vastanhog, was also susceptible. When the variety Samsun (intermediate) was crossed with the variety Daruma (very resistant), the F_1 progeny showed no water-soaking; whereas, some plants of the parental varieties water-soaked. Similarly, no water-soaking developed on the F_1 of the cross Havana 211 (intermediate) with Havana 142 (resistant). The failure to develop any water-soaking whatever in 2 trials on the F_1 of these two crosses is especially interesting in view of the fact that immunity is not involved in any of the parental varieties. This behavior suggests that the resistant character may be partially dominant. The results in the F_1 generation as a whole, however, indicate that the progeny are normally intermediate between the parents as far as such may be judged with a quantitative character.

Summarized results of an experiment with the F_2 progeny of the same crosses are also in table 2. The water-soaking scores for the different varieties do not cover as great a range as those with the F_1 progeny. This may be due in part to considerable variability between replications. The F_2 data are intended to show only that the amount of water-soaking in the F_2 generation of the several different crosses corresponds closely to that in the F_1 generation and to that which may be expected on the basis of parental behavior.

Although the results of the variety tests may be regarded as sufficient proof that the character of water-soaking is a heritable phenomenon, the limited F_1 and F_2 data in table 2 permit the conclusion that this character, although perhaps dependent upon many factors, is transmitted in crosses to succeeding generations according to expectations.

Good experimental data have not been secured for segregation or greater

variation in the F_2 than in the F_1 generation or in the parents. The number of plants that would be required would be impracticable for limited greenhouse space and moist-chamber facilities. It was believed that the time and space available might be more profitably applied to studies on the F_3 generation produced from selfed F_2 plants selected at random. Observational evidence on the individual F_2 progeny shown in table 2 was indicative of greater genetic variation in the F_2 than in the F_1 or in the parents. In the data this is only suggested by a relatively higher score and percentage of water-soaking in the F_2 than in the F_1, or in the parental types, in crosses involving the more resistant parents.

The F_3 generation. Three of the most promising crosses were grown in the field in the F_2 generation to supply seed of selfed plants for testing in the seedling stage: American (susceptible) × Daruma (resistant); American × Wisconsin Seedleaf (intermediate); and Wisconsin Seedleaf × Daruma.

TABLE 3.—*The percentage distribution in 5 classes of water-soaking secured in tests on three parent varieties and 290 F_3 families*

Designation	Number in test	Class (score) and percentage distribution[a]				
		0.25	0.75	1.25	1.75	2.25
American P_1	100 plants	3	18	44	28	7
Wis. Seedleaf P_2	100 do	20	36	41	3	.....
Daruma P_3	100 do	76	22	2	.....	.....
F_3 progeny ($P_1 \times P_2$)	110 families[b]	0.9	21.8	47.3	29.1	0.9
do ($P_1 \times P_3$)	95 do	13.7	40.0	33.7	11.6	1.0
do ($P_2 \times P_3$)	85 do	31.8	43.5	23.5	1.2	.....

[a] Based on the average of three scores from tests made at weekly intervals.
[b] Ten plants of each family tested.

Tests were made on approximately 100 F_3 families from each of the three crosses. Only 10 plants (selected at random) of each family could be tested, but this number was divided between two flats. It was necessary also to perform these tests in four separate series; consequently, one series was not strictly comparable with another. The three parental varieties were, however, used as controls in each series. The results of these tests are in a distribution table (Table 3) of average scores for the parents and the groups of families in each cross. In the F_3 generation, as in the F_1 and the F_2, the parental influence is in the expected direction. Referring to the class-center score of 0.25 (high resistance), for example, only 3 per cent of the variety American and 20 per cent of Wisconsin Seedleaf, but 76 per cent of Daruma, fell into this class. Correspondingly, 0.9, 13.7, and 31.8 per cent of the three crosses fell into this class in the order of expectation.

On the basis of percentage distribution (Table 3), there is evidence of greater variability in the F_3 progeny of crosses between resistant and susceptible parents than in that of the respective parents. The data, however, are not strictly comparable for parents and progeny because the former are

based on individual determinations and the latter on the average of 10 plants. Nevertheless, there is a greater spread, with no peaks, in the F_3 progeny of the two crosses involving the resistant parent Daruma than in the resistant parent itself where 76 per cent of the plants fell into the most resistant class (score 0–0.5).

These results are deemed sufficient to demonstrate that it should be possible to transfer the resistant characters to varieties of tobacco susceptible to natural water-soaking through breeding and selection.

Correlation between natural water-soaking and infection with tobacco wildfire

After it was evident that certain varieties of tobacco were more susceptible to natural water-soaking than others, it became important to determine whether these varieties were also more susceptible to wildfire under conditions favorable for the development of natural water-soaking. The results of two experiments with the three varieties, American (susceptible), Wisconsin Seedleaf (intermediate), and Daruma (resistant), are in table 4. Plants

TABLE 4.—*Results of two experiments showing relative reaction of three varieties of tobacco to water-soaking, together with their relative susceptibility to wildfire infection following inoculation*

Variety	Water-soaking score[a]			Number of wildfire lesions on a plant[a]		
	Exp. 1	Exp. 2	Ave.	Exp. 1	Exp. 2	Ave.
American	2.35	2.60	2.47	6.9	30.2	18.5
Wisconsin Seedleaf	1.85	1.85	1.85	4.5	20.9	12.7
Daruma	0.55	0.80	0.67	0.9	6.9	3.9

[a] Twenty plants of each variety in each experiment.

of these varieties were atomized from a distance of one foot or more with a water suspension of a pure culture of the bacteria, after which they were left in the moist chamber to permit water-soaking. The relative amounts of water-soaking developing after 48 hours' exposure in the moist chamber, and the relative amounts of wildfire (number of lesions) developing on the same plants 8 days from the time of inoculation, are shown in table 4. In these data, which include results of two separate trials, there is a direct correlation between the susceptibility of these varieties to water-soaking and their susceptibility to wildfire under the same conditions. In another similar trial, using plants two weeks older, most plants of the variety Daruma did not water-soak; whereas, all plants of both the variety American and the variety Wisconsin Seedleaf were rather heavily water-soaked. Most plants of Daruma likewise remained free of wildfire following inoculation; however, many lesions on a leaf developed on plants of American and Wisconsin Seedleaf. This difference in susceptibility is illustrated (Fig. 3) by representative leaves of the resistant variety Daruma and the susceptible variety American. The periphery of the leaf, which is usually the first to show

evidence of water-soaking and often develops relatively large water-soaked areas, also develops the greatest number of and the largest wildfire lesions (Fig. 3, B). The correlation of water-soaking susceptibility and wildfire susceptibility was also evident from inoculation experiments with several species of tobacco. The results of these tests are not presented.

During the spring of 1943 and 1944, outdoor plant-bed experiments were conducted. Seed of several varieties were sown about May 1 in replicated 2 by 3-foot sections of the plant bed. The beds remained under muslin-cloth

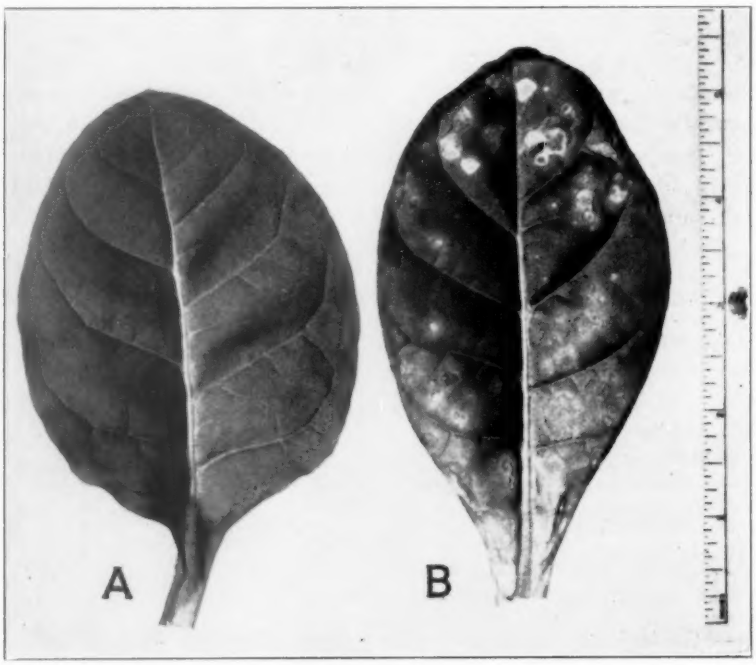

FIG. 3. Seedling tobacco leaves of two varieties, exposed to favorable conditions for water-soaking followed by artificial inoculation with the wildfire organism. A. Daruma, remained healthy. B. American, diseased.

cover most of the time, especially during periods of rain to reduce the possibility of storm water-soaking. The small tobacco seedlings were inoculated one month after sowing by watering with a suspension of powdered wildfire-infected leaf material.

The season in 1943, being unusually wet, proved to be very favorable for the trial. During the 3 weeks following inoculation on June 1 a total of 2.06 inches of rain fell, with at least a trace of precipitation during 14 days of this period. Records of relative humidity, both inside the covered plant bed and nearby outside, were taken from May 15 until June 16. A relative humidity between 95 and 100 per cent was maintained for at least 10 hours a day during 23 of the 32 days of the record as compared to only 2 days during the same period outside the plant bed. The longest period (51 hours)

of continuous, high relative humidity was recorded in the plant bed from 3:00 P.M. June 5 to 6:00 A.M. June 7, being continuously above 85 per cent and most of the time above 95 per cent in the absence of continued precipitation.

Extensive natural water-soaking occurred and numerous wildfire lesions began to appear on the more susceptible varieties about one week after inoculation. The severity of the disease gradually increased during the following two weeks. About 4 weeks after inoculation, when the notes (Table 5) were

TABLE 5.—*Results in outdoor plant beds during 1943 and 1944, comparing 15 varieties of tobacco for relative susceptibility to water-soaking and wildfire following artificial inoculation. Average of 2 replications*

Variety	1943			1944		
		Wildfire			Wildfire	
	Water-soaking score (40 plants)	No. lesions on a leaf[a] (20 plants)	Disease[b] score (20 plants)	Water-soaking score (40 plants)	No. lesions on a leaf[a] (100 plants)	Percentage diseased plants (200 plants)
Wisconsin Seedleaf (Strain T)	1.6	24.0	100.0	1.9	14.4	100.0
American	1.5	34.1	46.4	1.4	6.9	99.5
Vastanhog	1.1	28.2	54.4	1.4	8.5	97.5
White Burley (Judy's Pride)	1.0	14.1	32.1	1.4	4.3	90.0
Pennsylvania Broadleaf (Slaughter)	0.9	10.9	18.2	0.3	1.6	71.0
Maryland Broadleaf	0.8	28.9	36.6	0.3	4.6	93.0
Turkish (Samsun)	0.7	1.2	1.2	0.5	7.5	98.5
Maryland Mammoth	0.7	13.9	79.9	.....	.....	.....
Connecticut Broadleaf	0.6	19.0	39.7	0.9	6.9	97.0
Var. atropurpurea	0.5	10.4	16.0	0.9	7.3	98.5
Havana 211	0.2	26.2	44.6	0.6	15.8	100.0
Havana 142	0.2	14.9	20.9	0.0	7.1	97.5
Yellow Pryor	0.1	1.5	1.6	0.0	1.4	53.0
Orinoco (White Stem)	0.0	2.0	2.3	0.0	1.6	64.0
Daruma	0.0	1.8	2.4	0.0	1.3	52.0

[a] Counts made only on the leaf with greatest number of distinct lesions (halos).

[b] A score based on number of leaves on a plant completely destroyed by the disease and on percentage of leaf area necrotic as well as on number of lesions.

taken, plants of certain varieties were either killed or severely stunted and other varieties had just a few isolated lesions. The lesions were counted on single leaves from 20 plants of each variety and a disease "score" computed which also included an estimate of the amount of necrosis on these plants. The score for the most susceptible variety was then placed at 100 and the other scores were adjusted accordingly. The notes on water-soaking (Table 5) were taken 11 and 14 days after inoculation. Water-soaking occurred on other days, but detailed varietal comparisons were not made.

The 1944 plant-bed experiment was conducted in the same way as in 1943 except that the bed was located on muck soil rather than on silt loam soil and the young seedlings were inoculated 3 times at 4-day intervals rather than just once. The relative susceptibility of the varieties tested, both to water-

soaking and to wildfire, was approximately the same in the two years. The varieties Daruma, Orinoco, and Yellow Pryor proved to be very resistant to both water-soaking and disease. These varieties in 1943 and in 1944 developed only about 1/20 and 1/10, respectively, the number of lesions found on the more susceptible varieties (Table 5). About 44 per cent of the plants of these resistant varieties were free of disease in 1944; whereas, 100 per cent of the plants of Wisconsin Seedleaf and Havana 211, another Wisconsin variety, were diseased.

The results secured on tobacco varieties with wildfire are believed to be equally significant in relation to varietal differences in resistance and susceptibility to the closely related (4) blackfire disease under similar environmental conditions.

Variation in varieties of other plant genera

Tomatoes. A comparison was made on the water-soaking behavior of 17 commercial varieties or strains of tomato under moist-chamber conditions.

TABLE 6.—*Results showing the relative susceptibility of seven varieties of tomatoes (Lycopersicum esculentum) to water-soaking when placed under favorable conditions for its expression*

Variety	Water-soaking score[a]			Percentage of plants water-soaked[a]		
	Exp. 1	Exp. 2	Ave.	Exp. 1	Exp. 2	Ave.
Landreth's Bloomsdale	2.6	2.2	2.40	100	100	100
Golden Globe	2.4	2.1	2.25	100	100	100
Clark's Early	2.4	1.8	2.10	100	94	97
Early Baltimore	1.9	1.8	1.85	100	100	100
Harris' Extra Early	0.8	1.5	1.15	83	94	89
"Pearson"	0.9	1.1	1.00	92	88	90
Snow Ball or Albino	0.8	0.9	0.85	64	81	73

[a] Data based on two exposures in chamber, with six to eight plants of each variety.

In a preliminary experiment, 6 potted plants, 8 to 12 inches in height, of each variety were compared, using a sandy loam soil low in available potash. Later, 8 potted plants of each of 7 varieties—those showing the greatest range of variation in the first experiment—were further tested using compost soil, which is not as favorable to natural water-soaking as soil of lower fertility. Two tests were made with each group of plants at 10-day intervals and the results averaged.

Summarized data are in table 6 for the 7 varieties included in both experiments. The variety Bloomsdale was the most susceptible, with an average score of 2.40; and the variety Snow Ball was the most resistant, with an average score of 0.85. The percentage of plants water-soaked varied only from 100 to 73 per cent for the two varieties respectively. Varieties of tomato are, in general, more susceptible to natural water-soaking than are varieties of tobacco.

Oats. Preliminary trials were made on the relation of natural water-

soaking to infection with halo-blight [*Phytomonas coronafaciens* (Elliott) Bergey *et al.*] of oats in the seedling stage. Six varieties or selections were used, representing a range of reaction to the disease on the basis of field observations by Dr. H. L. Shands of the Dept. of Agronomy at the Wisconsin Station.

Twenty-five seeds of each variety were planted in rows across each of 6 flats (14 by 20 by 5 inches) containing a 3:1 mixture of greenhouse compost and sand. Oat seedlings were easily water-soaked, sometimes within an hour after placing in the moist chamber. Water-soaking was also observed, when the air and soil moisture were sufficiently high, on plants placed outside. Four of the replicated flats were inoculated by atomizing with a water suspension of bacteria at a distance of one foot or more from the seedlings so that the organisms should not be forced into open stomata.

Distinct varietal differences were found both in susceptibility to water-soaking and to disease. Summarized results are in table 7. The average

TABLE 7.—*Results showing the relation of water-soaking to susceptibility to halo-blight (Phytomonas coronafaciens) of varieties and selections of oat seedlings*

Designation	Water-soaking score (average)	Halo-blight reaction	
		In greenhouse following artificial inoculation	In field following natural infection[a]
X217	3.8	Very susceptible	Susceptible
Marion	3.5	Susceptible	Susceptible
X216	3.0	Susceptible	Moderately resistant
Vicland	2.5	Moderately susceptible	Moderately susceptible
C.I. 4007	1.4	Resistant	Resistant
C.I. 4006	0.9	Resistant	Resistant

[a] Rating by Dr. H. Shands of Department of Agronomy.

water-soaking score, using the range of 0 to 4 as for tobacco, varied from 0.9 for the variety C.I. 4006 to 3.8 for the variety X216, and resistance to disease was directly correlated with resistance to water-soaking. The lesions on the resistant varieties were smaller and fewer as compared to lesions on the susceptible varieties, some of which were very elongated, corresponding somewhat to previous water-soaked areas on the same leaves. The varietal reactions following artificial inoculation were approximately the same as those observed by Dr. Shands following natural infection (Table 7). The results suggest a reliable and rapid means of selecting and developing varieties resistant to the halo-blight disease.

Corn. Preliminary trials on water-soaking were also conducted with 17 dent-corn inbreds, 8 of which were selected to represent the range of susceptibility to Stewart's wilt [*Phytomonas stewartii* (Smith) Bergey *et al.*] as reported by Elliott (10). Twenty plants of each inbred were grown in flats for these trials.

Corn seedlings are very susceptible to natural water-soaking. Frequently, seedlings water-soaked in the greenhouse without being in the moist

chamber; whereas, this rarely occurred with tobacco, tomato, or oats. Differences in susceptibility between inbreds were not easily determined. Numerous droplets of moisture adhered to the leaves, making observations more difficult and uncertain, and water-soaking was somewhat obscured by the dark green color of certain inbreds. Consequently, the data on varietal variation in corn are not presented in detail. More data would be required to determine which inbred is most resistant to water-soaking.

Two of the inbreds, KYS and 32B, were consistently more susceptible to water-soaking than were the others. KYS is the most susceptible to wilt of 50 selections that were tested by Elliott (10). Leaves of apparently healthy plants of these two varieties water-soaked so frequently, just standing on the greenhouse bench, that they became necrotic. This necrosis may have been associated with the entry of weak parasites into water-soaked tissue as suggested by Johnson (15) for other plants, or it may be due to an accumulation of toxic concentrations of salts following frequent guttation and drying as suggested by Curtis (9).

Differences in susceptibility to water-soaking of inbreds, with both seedling and nearly mature plants, have been observed on plantings made outdoors. For example, in one trial with 40 seedlings of each of two inbreds, no plants were water-soaked of the inbred Ohio 67, which is moderately resistant to wilt; whereas, all plants were water-soaked of KYS, which is very susceptible to wilt.

The results, although limited in extent, suggest that water-soaked tissues may influence infection with and development of the Stewart's wilt disease.

DISCUSSION

The results have demonstrated that varieties of *Nicotiana tabacum* and other plants possess varying degrees of resistance to natural water-soaking. The variation secured was of such magnitude that it was believed to offer opportunity for the study of the inheritance of this character in plants. It seemed especially desirable to determine whether resistance to water-soaking might be introduced into susceptible commercial varieties with the purpose of improving their resistance to specific diseases. A part of the present investigations was to determine the correlation between natural water-soaking and resistance to disease, especially as applied to tobacco wildfire. Fairly good agreement was found, which not only supports the earlier conclusions from this laboratory that water-soaking predisposes plants to disease, but suggests a shorter and perhaps improved method for selecting plants for disease resistance. It may be easier, for example, to eliminate varieties or plants susceptible to certain diseases by first determining their relative susceptibility to water-soaking. The technique may be particularly useful in breeding for resistance to various bacterial leaf-spot diseases that occur infrequently in natural epidemics or that are difficult to reproduce experimentally in the greenhouse. The major contribution of this investigation is, therefore, the suggestion it offers for continued efforts with numerous other hosts and diseases.

Considering the entire data on the F_1 and F_2 generations of twelve crosses between tobacco varieties varying in response to natural water-soaking, as well as the results on F_3 progeny of three of these crosses, the genetic behavior is best explained on the basis of multiple factors segregating in a normal manner. There is some evidence, however, of the existence of dominant factors for resistance. The most significant fact from the genetic studies is that crosses may be made with commercially important susceptible varieties with some assurance that lines resistant to water-soaking may be secured.

Some difficulties and problems arise in an investigation of this sort involving a special technique to secure the expression of a genetically new character that is easily influenced by non-genetic factors. There is no certainty, for example, that the varieties used were homozygous for the character studied, or that a very considerable part of the variation secured within a variety is a normal variation due to uncontrollable variation in the environment. It is certain that the expression of the natural water-soaking character is very delicately balanced with the environment. Consequently, minor modifications may prevent its appearance and greatly alter the severity of disease.

Questions of proper or satisfactory terminology are also justifiable in connection with the genetic behavior of natural water-soaking. The terms "resistance" and "susceptibility" to natural water-soaking have been used in this paper. This terminology is in a measure confusing when it is used in close association with their long-established use as applied to genetic variations in host behavior toward pathogens. Proper terminology for future use is therefore sought.

SUMMARY

The natural water-soaking reactions of several foreign, domestic, and local varieties of tobacco have been compared under moist-chamber and outdoor plant-bed conditions. The amount of water-soaking is expressed as (1) a "score" based on the amount of leaf area water-soaked, and (2) the percentage of plants water-soaked. One moist-chamber test yielded, for example, differences in score ranging from 2.36 to 0.09 and in plants water-soaked from 100 to 8 per cent respectively. The susceptibility of varieties was greatly influenced by environmental factors; however, the relative differences between the varieties remained approximately the same.

The inheritance of the natural water-soaking character in crosses involving seven varieties ranging in reaction from the highly resistant to highly susceptible has been studied. The F_1 progeny of most crosses were apparently intermediate between the parents; however, when some varieties were crossed, there was evidence of partial dominance of resistance. The reactions of the F_2 and F_3 families clearly indicate segregation of genetic factors controlling the inheritance of this character. Many genetic factors are apparently involved. F_3 families approaching the resistance of the most resistant parent were obtained.

Varieties of tobacco likewise showed varying degrees of resistance to wildfire (*Phytomonas tabaci*) when inoculated under conditions favorable for the occurrence of natural water-soaking. Results in greenhouse and moist-chamber experiments were essentially the same as in experiments in outdoor plant beds. Resistance to this disease was closely correlated with resistance to water-soaking.

Variation in reaction to natural water-soaking was also found between different species of *Nicotiana* and between varieties of tomato, of oats, and of corn. Varieties of oats inoculated with the halo-blight organism also showed correlation of water-soaking and disease.

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THE NEMATOCIDAL AND FUNGICIDAL VALUE OF D–D MIXTURE AND OTHER SOIL FUMIGANTS

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Until recently, the most effective soil fumigant, other than steam, for the control of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, and of most soil fungi, was chloropicrin. Taylor (6) has reviewed the literature pertaining to control of nematodes by this material and Leukel (2) lists a number of investigators who have found chloropicrin to be an effective fungicide. In April, 1943, Carter (1) reported on a new material, D–D mixture for short, which had shown great promise as a "soil amendment and disinfestant" in the Hawaiian Islands. Three years' testing under tropical conditions showed that D–D mixture injected in soil at intervals one foot apart, at 150 to 200 pounds per acre, without benefit of subsequent cover, gave good control of a soil complex including Anomala beetle larvae (*Anomala orientalis*), the root-knot nematode (*H. marioni*), and Pythiaceous fungi (presumably *Phytophthora cinnamomi*, *P. parasitica*, and other members of the Pythiaceae that attack pineapple in Hawaii). Carter considered that D–D mixture was at least the equal of chloropicrin in its benefits and, furthermore, it was lower priced and simpler to handle. Pinckard (4) subsequently reported the value of D–D mixture as a nematocide.

EXPERIMENTS

The experiments reported here were begun in July, 1943, to test the nematocidal and fungicidal properties of D–D mixture and were continued in their various phases until September, 1944. Both forms of D–D mixture were studied, the redistilled mixture of approximately two parts of 1,3-dichloropropylene and one part of 1,2-dichloropropane, and the crude mixture of approximately two parts of 1,3-dichloropropylene, one part of 1,2-dichloropropane, and one part of 3-carbon-atom compounds of trichlorides and tetrachlorides. In addition two other materials similar in chemical nature, monochlorobutenes and trichlorobutanes were studied. These materials were supplied through the courtesy of their manufacturer, the Shell Development Company, Emeryville, California. In preliminary tests, chloropicrin[1] was compared with the other products, but in later trials it was not included.

The root-knot nematode (*Heterodera marioni*) was the only nematode studied; fungi studied were *Rhizoctonia sp.* (probably *R. solani*), *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen, *Fusarium sp.* (probably *F. martii*) and *Pythium aphanidermatum* (Edson) Fitzpatrick.

The soils used varied from Norfolk loamy sand, well drained, with or

[1] The chloropicrin was supplied by Innis, Speiden and Co., 117 Liberty Street, New York, as "Larvacide."

without a fairly high organic matter content, to Woodstown silt loam. In a single test, a silt loam, which had been used in greenhouse culture for 8 months and was well mixed with rotted leaves, was studied. Experiments were performed outdoors during the fall of 1943, the winter of 1943–44, and the spring and summer of 1944, as well as in a greenhouse during the fall of 1943 and winter of 1943–44.

In early outdoor tests and in all greenhouse trials the D–D mixture, or other materials used, was injected in holes 3–4 inches deep (made by hand with a sharp stick), one hole in the center of each square foot of surface. The points of application were not staggered. This method is slower than the standard procedure described by Taylor (5), because the application points are more numerous and the rate of chemical per injection hole is less for an equal poundage of chemical per acre. In later tests, Taylor's spacing and rate of chemical per injection hole were adopted; the injections were 12 inches apart in staggered rows, the rows 12 inches apart, the holes 6 inches deep. In still other tests, the rows were 18 or 36 inches apart with injection points in the row 12 or 18 inches apart.[2] The chemicals were measured and injected by hand, using a burette and a flask with a side tube. With one exception, that of a trial in which a chloropicrin-treated plot was compared with a D–D-treated plot (Experiment No. 1), the treated soil was not covered; sometimes the soil was well wetted down after treatment, sometimes it was left undisturbed.

Crops, either seeded directly or as transplants, were started in out-door treated soil 2–3 weeks after chemicals were applied, or as soon as convenient for cooperating farmers on whose lands the tests were installed. In the greenhouse, pea and spinach seed, the two test plants grown, were sown at intervals ranging from 1 to 21 days after the soil was treated. All greenhouse tests were in raised benches, one foot deep, 12 feet long, and either 3 or 6 feet wide.

The control of root-knot achieved by the D–D mixture or other soil amendments was judged either visually, by comparing the roots in gross characters with plants grown alongside in untreated soil, or by actual count of the number of galls on representative plants grown in treated and untreated soils respectively. Soil temperatures at 6-inch depths were taken when feasible.

Studies on Root-Knot

Experiment No. 1 consisted of 18 plots, each 3 by 5 feet, arranged linearly in a concrete container 6 feet wide, 2 feet deep, and 75 feet long, filled with a silt loam mixed with rotted leaves as used in a greenhouse in which cucumbers and tomatoes had been grown during the preceding winter and spring. This soil was heavily infested with *Heterodera marioni*. The chemicals, D–D mixture (both crude and purified), chloropicrin, monochlorobutenes, and trichlorobutanes, were injected into 3 replicated areas of 15 square feet each, and the treated areas were separated by an untreated space one foot wide

[2] Suggestion from G. H. Godfrey; personal correspondence.

to hinder, if not prevent, inter-plot diffusion of the fumigants. Injections were at the rate of 150 pounds per acre in holes 3 to 4 inches deep, one injection point for every square foot. Appropriate areas left untreated served as checks. After treatment the surface soil was wetted down, and, in addition, plots with chloropicrin injections were covered with a double layer of wet gunny sacking. Plots were treated on July 1–3 (1943), and two weeks later tomato seeds were sown. The odor of the D–D mixture was still noticeable then, that of the other 3 chemicals could not be detected. Germination was good and there was no evidence of any toxic action by the D–D mixture. Seven weeks later (Sept. 3–4) the tomato plants were dug, the roots were examined for nematode galls, and the tops were weighed. Galls were not counted, but good control of the nematode by crude and by purified D–D mixture and by chloropicrin was obtained. No control was obtained with monochlorobutenes and trichlorobutanes. Check plants were severely galled. Mean weights of tomato tops (in grams) were 58 each for the check and monochlorobutenes, 78 for trichlorobutanes, 85 for purified D–D mixture, 99 for crude D–D mixture, and 114 for chloropicrin. (The difference necessary for statistical significance is 25 grams.)

Experiment No. 2 is reported here despite the fact that the soil used in Experiment No. 1 was used over again, a procedure not beyond criticism. The soil was removed from the container, well mixed, and replaced. On September 18 it was treated with D–D mixture, crude and purified, and with chloropicrin, 180 lb. per acre. Each plot had 25 square feet of soil and one injection point per square foot, and each treatment was in 3 replicates. Again, appropriate areas served as checks, and the treated areas were separated from each other by an untreated space one foot wide. As in Experiment No. 1, the soil was wetted down, but in Experiment No. 2 no plots were covered. Bush beans were selected as test plants, because of the advanced season, and sown in each replicate at 3 different dates, 11, 16, and 18 days after soil treatment. Sash were placed over the plots 6 days after the last planting and maintained *in situ* with appropriate regulation to suit outside temperatures. One month after the first planting the plants were dug and counted, and the roots were examined for root-knot. The data obtained are in table 1.

D–D mixture again gave good control of the root-knot nematode, but it caused some injury (expressed as reduced emergence[3]) to beans planted as long as 18 days after the soil was treated. All chloropicrin fumes apparently had been released at 16 days, but not at 11 days, after treatment.

Experiments No. 3 and No. 4 were in the same soil, in the same container, as Experiment No. 2. The 12 plots of Experiment No. 2 were left untouched,

[3] In another outdoor test, D–D mixture and trichlorobutanes, injected 6 inches deep into soil at 190 and 380 pounds per acre respectively, have injured tomatoes when transplanted 14 days after treatment. The soil was a poorly drained Woodstown silt loam of relatively low organic matter content and pH 5.5; the mean minima and mean maxima soil temperatures, 6 inches deep, were 71° and 80° F. respectively. No injury was noted in this soil when monochlorobutenes were simultaneously injected at rates up to and including 1,000 pounds per acre.

TABLE 1.—*Relative degree of control of Heterodera marioni in infested soil treated with D–D mixture (crude and purified), with chloropicrin, or left untreated. Rate of application, 180 pounds per acre. Indicator crop was bush bean*

Treatment of soil	Nematode attack	Germination of beans sown at different times after treatment of soil		
		11 days	16 days	18 days
		Pct.	*Pct.*	*Pct.*
Untreated	Severe	82.5 ± 6.1	84.3 ± 4.3	82.6 ± 4.3
D–D (crude)	Slight to moderate	68.0	77.7	74.5
D–D (purified)	Slight to moderate	64.0	79.5	76.0
Chloropicrin	Moderate	65.0	89.2	83.6

with weed growth unhindered and abundant, until February 10, 1944, when weeds were pulled, and the soil chopped with a hoe, care being taken to keep the soil in each plot in place. On February 25, Irish potatoes were planted, 20 per replicate, and grown without benefit of fertilization until harvested on June 13. On May 9 potatoes growing in soil into which D–D mixture had been injected were darker green and of more uniform size than plants growing in control soil. The heights of all plants were taken: plants growing in soil treated with D–D mixture averaged 11.5 inches, while those in untreated soil averaged 8.5 inches, a difference that is statistically significant. Plants growing in chloropicrin-treated soil averaged 10.8 inches tall. There was little difference in yields of tubers from different plots; nematode infection was light in check plots and relatively sparse on tubers from treated plots. The roots themselves were not examined.

In Experiment No. 4, four rows of Lima beans, 25 seeds per row, were planted on June 18 in each plot used in Experiment No. 3. The soil had been kept in place except for the small amount of hoeing necessary to prepare a seed bed. On August 8, eight representative plants were removed, with the roots intact, from each plot, the soil was washed away, and the nematode galls were counted. The counts (Table 2) show that for 9 months,

TABLE 2.—*Effect of treating root-knot nematode infested soil with D–D mixture and with chloropicrin; mean number of galls on roots of Lima bean plants grown in treated and untreated soil, nine months after soil was disinfested. Eight plants from each plot were examined*

Plot No.[a]	Treatment of soil	Mean number of nematode galls	Plot No.	Treatment of soil	Mean number of nematode galls
1	D–D[b]	0.3	7	Chloropicrin	238.3
2	None	524.3	8	None	325.5
3	D–D	0.0	9	D–D	30.7
4	Chloropicrin	44.1	10	Chloropicrin	149.0
5	D–D	0.3	11	D–D	3.3
6	D–D	0.0	12[c]	None	.....

[a] Plots 1 and 2 were at one end of the concrete container.

[b] Both crude and purified D–D mixtures were used; since results were the same with both, no distinction is indicated.

[c] Plot 12, an untreated check, was abandoned because of its proximity to a drainage pipe that caused flooding of the plot.

with no precautions taken to prevent interplot movement of soil by rain or irrigation water, and with weed growth unhindered for at least 4 months, a root-knot nematode population, reduced by the use of D–D mixture, had maintained its low level. Where chloropicrin was used, the nematode population, once markedly reduced, had increased, but had not reached its former level. It should be noted here that the manufacturers of chloropicrin generally recommend that their fumigant, when used against nematodes in the soil, be applied at a much higher rate per acre than was used in these tests.

Experiment No. 5 consisted of long, paired beds of well-drained Norfolk loamy sand, with moisture-holding capacity approximately 11 per cent, pH approximately 6.5, and organic matter content high and so maintained for the past 20 years by the owner of the land. Root-knot had caused some losses in this general area in 1943 (3). The beds were made up for celery, to be grown under sash. Each bed was 4 feet wide and over 200 feet long; in these tests 90 linear feet per bed were used. The 3-foot space between each pair of beds was occupied by the irrigation pipe of a Skinner irrigation system, and two beds not included in the experiment separated each pair of beds studied. One bed of each pair was treated with D–D mixture, there being 3 rates of application per bed, 120 square feet of soil for each application rate. The untreated bed of each pair served as check. On January 26 or on February 16, 1944, the D–D mixture was injected at depths of 4.5, 7, and 8 inches, at points 12 inches apart in staggered rows, and injections were at rates of 125, 190, 200, 250, 325, and 400 pounds per acre. The soil was left uncovered until March 13–15, then celery transplants, free from *Heterodera marioni,* were set out, and sash were placed in position. The sash were regulated to suit outside temperatures until permanent removal on April 20, 1944. The actual temperatures at 6-inch soil depth in these months are not known, but we do have access to soil temperatures at this depth, taken in the same area, for a 5-year period, 1932 to 1936. Mean soil temperatures for January to May inclusive may have been approximately as follows, except that the soil temperatures would have been higher as soon as sash were placed over the celery plants: January, maximum 38° F., minimum 34° F.; February, 40° F. and 36° F.; March, 47° F. and 41° F.; April, 64° F. and 57° F.; and May, 81° F. and 72° F.

No injury that could be ascribed to the presence of the D–D mixture was noted. On June 6, when the grower on whose land the test was installed was harvesting his crop, 60 plants were pulled from the center of each treated area and a similar number from the adjacent check plot, the soil was shaken from the roots, and the celery was weighed without trimming. Five other plants, chosen at random, were removed at the same time from each treated and untreated area, with care to preserve the roots as intact as possible. The soil was washed from the roots, and the nematode galls of each root system were counted. Results (Table 3) show that D–D mixture reduced the nematode population, but the yields from treated and untreated areas of soil are little different.

Experiment No. 6 was in the same type of soil and on the same farm as Experiment No. 5, the tests being 200 yards apart. The area was one in which celery was diseased severely with root-knot in 1943, yields being reduced 22–36 per cent in untrimmed plants and 24–48 per cent in trimmed celery (3). Two rectangular and parallel areas of infested soil, each 4 feet wide and 150 feet long, on either side of an irrigation pipe of a Skinner irrigation system and separated by a 4-foot width of soil, were selected, and into one strip D–D mixture was injected on February 26, 1944, while none was injected into the companion strip. The method of application was as described for Experiment No. 5, with rates of application 210, 290, and 375

TABLE 3.—*Average numbers of nematode galls on, and yields of, celery grown in soil infested with Heterodera marioni and treated with D–D mixture or left untreated*

Treatment (pounds D–D mixture per acre)	Depth of application (inches)	Date of application (1944)	Mean number galls per plant	Mean yield per plant (pounds)
125	7	Jan. 26	36.1	1.16
0			114.1	1.17
190	7	do	8.0	1.04
0			490.1	1.13
250	7	do	0.9	0.77
0			365.2	1.07
200	8	Feb. 16	4.2	0.78
0			> 1000.0[a]	0.77
325	8	do	0.0	0.81
0			273.2	0.92
400	8	do	0.0	1.15
0			820.4	0.92
200	4.5	do	0.2	0.86
0			556.8	0.93
325	4.5	do	0.8	1.07
0			132.2	1.20
400	4.5	do	0.0	1.14
0			156.4	1.26

[a] After one thousand galls had been counted each plant was discarded.

pounds per acre, respectively, and 2 replications of each rate. Injection depth was 6 inches. The treated area was 80 square feet per replicate. The treated soil was not covered. Lettuce transplants, free of *Heterodera marioni*, were set out in treated and control areas on March 25. On or around April 20 it was noted that plants growing in treated soil were smaller and darker green than adjacent plants in untreated soil; the higher the rate of application, the greater the injury. Later, the lettuce recovered except where application rate was 375 pounds per acre. Due to a misunderstanding, the grower cut all marketable heads of lettuce from treated and untreated areas on May 22. No weights were taken by him. Two days later, the writer counted the number of heads that had been harvested and found that 63 per cent were removed from untreated plots, and 70, 65, and 30 per cent from plots in which the soil had been treated with D–D mixture at 210, 290, and 375 pounds per acre respectively.

The root systems of 6 plants taken at random from each replicate were carefully removed, the soil was washed away, and the galls were counted. Plants grown in untreated soil averaged 660 galls per plant, while those grown in treated soil, regardless of the rate of application, averaged 3 galls per plant. Control of the root-knot nematode was as good at 210 pounds per acre as at the higher rates.

In Experiment No. 7, D–D mixture was injected as in Experiment No. 6, but at 275 and 550 pounds per acre, respectively, into Norfolk loamy sand, medium to well drained, organic matter fairly high, moisture-holding capacity 18–20 per cent, pH 6.0–6.2. This soil was known to be infested with *Heterodera marioni,* and was shown subsequently to be infested also with the fungus, *Pythium aphanidermatum.* The soil was treated on April 18, 1944, there being 2 replications of each rate per acre. Each replication consisted of 360 square feet. Adjacent untreated areas served as checks. On June 27, snap beans were planted, 4 rows in each treated and untreated area. On July 27, 40 plants, taken at random, were removed from treated and untreated areas with root systems intact, the soil was washed away, and the nematode galls were counted. One hundred and ten galls (mean) were found on the roots of plants grown in untreated soil, while no galls could be found on the roots of plants grown in the treated soil. Once again D–D mixture was an effective nematocide against *Heterodera marioni.*

Studies on Fungi

From repeated greenhouse tests, during the winter of 1943–44, in Woodstown silt loam infested with a *Rhizoctonia-Fusarium* complex capable of causing pre-emergence and post-emergence damping-off of spinach and pre-emergence damping-off (seed decay) of peas (*Pisum sativum*), it has been found that D–D mixture at rates up to 1,000 pounds per acre, trichlorobutanes at 250 pounds per acre, and monochlorobutenes at 450 pounds per acre, have very little value in the eradication of these fungi from an infested soil. Simultaneously, in the same soils, Arasan (2 per cent by weight) on spinach, and Spergon (2 per cent by weight) on peas, gave good to excellent control of damping-off. Soil treated with D–D mixture at 1,000 pounds per acre gave the first indication of reducing damage by damping-off fungi when 18 per cent more plants emerged from soil treated with the D–D mixture than from the untreated check soil, but Arasan used simultaneously reduced the damping-off from approximately 60 per cent in the check to less than 10 per cent when the seed treatment was used. Seed treatment is faster and easier, and consistently more effective. In these greenhouse tests the soil moisture was maintained at its optimum, and the pH ranged from 6.0 to 6.5. Soil temperatures 4 inches deep ranged from a minimum of 60–66° F. to a maximum of 61–78° F. Little phytocidal action by the D–D mixture was noted on germinating spinach planted 6 days after soil treatment, and none on spinach planted 14 days after soil treatment. There was some evidence of injury to the peas planted 10, 12, 15, and 22 days after soil treatment. Inter-

estingly enough, the injury was not observed on peas planted 24 hours or 3 days after treatment.

Pythium aphanidermatum was found in the soil used in Experiment No. 7. In this test, designed to determine the value of D–D mixture as a nematocide at 275 and 550 pounds per acre, the fungus caused a 10 per cent post-emergence damping-off of the beans when the plants were 2½ to 3 weeks old. One month after planting, counts of surviving plants were made in areas treated with D–D mixture or comparable untreated areas. Assuming the rate of seeding to be the same in all rows, and there seemed to be no reason for not believing this to be so, it was found that a mean of 95 plants were present in 30 feet of treated row, and 97 plants were present in a similar length of untreated row. Though there exists the possibility of recontamination with *Pythium aphanidermatum* from outside sources, it would seem from this simple test that D–D mixture at 550 pounds per acre did not inhibit the action of the fungus.

TABLE 4.—*Mean number of tomato transplants, variety Marglobe, diseased with Fusarium wilt, growing in infested soil treated with D–D mixture before planting or left untreated. Sixty transplants set out per treatment and in checks*

Treatment (pounds of D–D mixture per acre)	Plants surviving transplanting		Plants with wilt	
	Number	Per cent	Number	Per cent
0	29	48.3	23	79.3
190	26	43.3	22	84.6
250	27	45.0	19	70.3
385	27	45.0	20	74.0
500	30	50.0	22	73.3
1000	34	56.6	24	70.5

Experiment No. 8 was designed to still further test the action of D–D mixture against soil fungi. A loamy sand, of low organic matter content and good drainage, 15–20 per cent moisture content, pH 5.5, which had just supported a crop of tomatoes almost 100 per cent diseased with *Fusarium oxysporum* f. *lycopersici*, was treated with D–D mixture at 190, 250, 385, 500, and 1,000 pounds per acre on July 10, 1944. The chemical was injected in holes 6 inches deep, at points 12 inches apart in staggered rows. There were 6 replications of each rate of application and, in addition, 6 check plots; each replicate was approximately 36 square feet. Two weeks after treatment of the soil, 10 tomato plants of Marglobe variety, free from root-knot, were set out in each replicate. Unusually hot weather caused a rather high plant mortality, but about 50 per cent survived; the plants' demise was not thought to be due to toxicity of D–D mixture. On October 15, stems of all plants were split and examined macroscopically for the discoloration indicative of infection with *Fusarium*. Enough isolations were made to check the belief that this discoloration was due to *Fusarium oxysporum* f. *lycopersici*. Findings (Table 4) show that D–D mixture, at rates up to and including 1,000 pounds per acre, applied to soil containing undecayed roots of tomato

plants that had died from Fusarium wilt, does not reduce the population of the pathogen as measured by the methods of the test. Taylor (6) found that chloropicrin, at 200 pounds per acre, did not control *Heterodera marioni* when the soil contained fresh infected roots, and gave only fair control at 300 pounds per acre. However, good control was obtained at 200 pounds per acre applied after the nematode-infected roots had disintegrated.

DISCUSSION AND CONCLUSIONS

In the experiments reported here, D–D mixture, at 150 pounds per acre, has been an effective nematocide against the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. As a fungicide, D–D mixture has given disappointing results, for at rates as high as 1,000 pounds per acre it has failed to control damping-off of spinach and pea seed, caused by *Rhizoctonia sp.* (probably *R. solani*) and by *Fusarium sp.* (probably *F. martii*). The two additional soil fungi, *Pythium aphanidermatum* (Edson) Fitzpatrick and *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen, were not measurably affected by the presence of D–D mixture in the soil at rates of 550 and 1,000 pounds per acre, respectively. Seed treatment was more effective, and easier to use, in the control of the damping-off organisms.

Two other materials similar in chemical nature, namely monochlorobutenes and trichlorobutanes, had no fungicidal action at rates up to and including 450 pounds per acre, and no nematocidal action at 150 pounds per acre. Trichlorobutanes had decided phytocidal action, D–D mixture less, and monocholorobutenes none at all in these tests.

D–D mixture may be applied to cold soil (38–40° F.) and its effectiveness as a nematocide does not seem to be impaired. However, at low temperatures and relatively high dosages per acre (375 pounds) a toxic residue may remain in the soil, and lettuce transplants have been injured in these studies.

The writer has found D–D mixture easy to use and he has suffered no discomfort from its use. Spilled on the skin of the hands, and immediately wiped off, but not always washed off with soap and water as recommended by its manufacturers until some hours later, D–D mixture has not burned the skin. Its fumes are not pleasant to breathe, but if not inhaled unnecessarily they occasion no great precautions in its use. It is best to work on the windward side of the container, but D–D mixture has been used on many occasions in a closed greenhouse where it caused no ill effects to humans or to plants such as tomato, spinach, and cabbage growing within range of the fumes liberated from the material. Monochlorobutenes is likewise not unpleasant to use; trichlorobutanes is a dark, heavy, sticky liquid, which is difficult to clean from glassware, and probably also from metallic injection machines. Chloropicrin fumes are unpleasant and may damage growing plants near treated soil in closed places such as greenhouses.

SUMMARY

D–D mixture has been found to be an effective nematocide against *Heterodera marioni* (Cornu) Goodey at rates as low as 150 pounds per acre.

No covering of the soil, other than a possible wetting of the surface, seems necessary for good retention of fumes of the chemical. In limited comparisons, in which bush, snap, and Lima beans, tomatoes, potatoes, and celery were used, D–D mixture was the equal of chloropicrin as a nematocide. Two materials chemically similar to D–D mixture, namely monochlorobutenes and trichlorobutanes seem to possess no nematocidal values at 150 pounds per acre.

D–D mixture seems to possess little value as a fungicide in soil disinfestation studies with the damping-off fungi *Rhizoctonia sp.* (probably *R. solani*) and *Fusarium sp.* (probably *F. martii*), and with *Pythium aphanidermatum* (Edson) Fitzpatrick, and *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen. Dosages as high as 550 pounds per acre were not fungicidal to *Pythium*, and dosages as high as 1,000 pounds per acre did not control the other fungi.

D–D mixture has a slight phytocidal action if plants are set out in treated soil too soon after the treatment. At 150 pounds per acre, no injury has been found if 2 weeks elapse between treatment and time of planting. In cold soils, the time interval for safety may be 3 weeks or longer; limited studies indicate that the time interval varies with the dosage and also with the particular plant used. Lettuce has been injured when celery was not damaged.

D–D mixture may be applied to cold soil (30–40° F.) and its effectiveness as a nematocide does not seem to be impaired.

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INHERITANCE OF RESISTANCE TO BARLEY STRIPE[1,2]

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INTRODUCTION

Barley stripe, caused by *Helminthosporium gramineum* Rabh., occurs in nearly all countries where barley is extensively grown, but is of importance only in the more cool and humid regions (6, 16).[3] In the United States infections have been usually less than 10 per cent, while reductions in yield of 5 per cent were fairly common.[4,5] Stripe is an important disease in the winter barley areas and the West Coast, especially California.[6,7] In the spring barley areas of the upper Mississippi Valley the widespread use of Wisconsin Barbless, a somewhat resistant variety, has reduced the prevalence of the disease in recent years.

The cycle of infection for barley stripe and pathogenesis in relation to disease development have been well worked out. The earlier work has been reviewed by Leukel *et al.* (6), and more recently Stelzner (14) has reported further on the host-fungus relations. Conidia are produced in abundance on leaves, leaf sheaths, and culms of infected plants at about the time normal healthy plants are flowering, and are carried by air currents to florets of healthy plants. Under favorable conditions the conidia germinate and as the kernel develops the mycelium becomes established on and in the pericarp and aleurone layers, but not in the embryo. The following season when the kernels germinate the mycelium penetrates the young seedling, becomes systemic, and eventually invades all parts of the plant. Infected plants are more or less reduced in size, depending on the variety, and seldom produce plump germinable kernels.

Stripe can be controlled by seed treatment with the organic mercury dusts or similar surface disinfectants. However, it has been found [reviewed by Shands and Arny (12)] after severe natural infection and artificial inoculation that a number of barley varieties are more or less resistant.

Artificial inoculation trials were started at Wisconsin, and were continued in connection with the barley breeding program. Since 1935 a large

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[2] The writer wishes to make grateful acknowledgment to Drs. H. L. Shands and J. G. Dickson for generous aid and counsel during the investigations and preparation of the manuscript. The figures were prepared by Eugene Herrling.

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number of varieties and selections have been severely tested. With the cultures of the fungus used, some varieties have remained highly resistant while others have shown various degrees of resistance and susceptibility (12).

The inheritance of resistance to stripe was studied by Isenbeck (5).[8] His type crosses and results may be summarized as follows:

Immune × immune—62 lines showed no infection in the F_3 generation.

Immune × susceptible—among 88 lines, 82 were stripe-free in the F_3 generation, 6 had low infection. Resistance was apparently dominant.

Resistant × resistant—some F_3 lines were more susceptible than either parent.

Susceptible × resistant—dominance of resistance was again indicated.

Susceptible × susceptible—the majority of the F_3 lines were susceptible, but some were more resistant than either parent.

Both wet and dry inoculations were used by Isenbeck. Only a single test was made on each line, and the reactions of the parents under comparable conditions were not indicated. A few lines had as much as 60 per cent infection. The large number of lines in all crosses which had no infection might be ascribed partly to the inadequacies of his inoculation techniques. In his results there were no sharply defined divisions between slight and high susceptibility, but rather continuous variation. He concluded that resistance was a heritable character, and that resistance was dominant in the varieties used. It was not possible to make a factorial analysis, although the absence of sharp divisions for infection groups suggested that several factors were involved. In one cross, susceptible × resistant, he used a "spore-kernel" inoculation method to test the lines, and found that the results agreed with those obtained by floral inoculation, but infection was more certain with the "spore-kernel" method.

The work of Shands *et al.* (13) indicated that factors for stripe reaction were inherited, but gave no suggestions as to the exact manner of their inheritance. From crosses involving resistant and susceptible parents, selections with various degrees of resistance and susceptibility were obtained. Backcrosses of resistant lines to the susceptible parent gave increased susceptibility.

Since resistance to stripe is important in the barley breeding programs in Wisconsin and other barley producing States, it seemed desirable to know more definitely the inheritance of the reaction to the disease. Attempts were made to determine how the resistance of certain varieties was inherited, and which chromosomes carried the factors for resistance.

MATERIALS AND METHODS

Inoculation Technique

The method of inoculation used in this work has been described (1, 12). It has given fairly consistent infection in susceptible varieties. The method

[8] Also reported by Roemer *et al.* (11).

has been tested at various stages to determine what conditions were optimum for infection, and where the schedule could be changed when necessary without seriously affecting the results. In general, inoculum 2 to 4 days old has been best, although that 12 days old has given good results. The amount of infection was increased as the incubation period was lengthened, but beyond 4 days the increased amount of infection was more than offset by a decrease in the total number of plants developing beyond the early seedling stage. Seed of rye and barley used as the substrate for growth of the inoculum gave no better results than wheat. A higher water content in the wheat medium has given some increase in infection, but again this was offset by a decrease in the total number of plants surviving.

Source of Causal Organism

The culture of fungus used in all the present experiments has been designated as C–1. This culture originated from a single hyphal tip isolated from a Wisconsin collection in 1932 (12). Since then it has been carried in artificial culture by mass transfers. It has maintained a high degree of pathogenicity on Oderbrucker, and other barley varieties have reacted to it in essentially the same manner from year to year. There is very little chance that natural infection has affected the results, since practically no naturally infected plants have appeared on the Hill Farms during the time these experiments were carried out.

Parental Varieties

The stripe reactions of the varieties and selections used in the inheritance studies are in table 1. The reactions are given as averages only, but indications of the variability between tests are presented in figures 1, 2, 4, 5, and 6. Varieties in which there have been no stripe-infected plants, such as Persicum and Brachytic, have been classified as highly resistant. Varieties with infections from 2 to 15 per cent, such as the Lion selections, have been considered resistant. Oderbrucker, Colsess IV, and Iris have been highly susceptible under the conditions of these tests. In the results and discussion that follow these terms will be used in the sense indicated.

Lion is a black barley which has been used as the smooth-awned parent for several varieties grown commercially in the United States. The two selections were alike in all respects, except that Lion 28 has seemed to be slightly more susceptible to stripe than Lion 36. Persicum is a smooth-awned, 2-rowed variety that came from Russia. Brachytic, a selection with much shortened internodes, originated as a mutant in normal Himalaya (8). Oderbrucker is a rough-awned variety which was the standard malting barley in Wisconsin for many years. Robertson (9) developed the stock Colsess IV, which carries the lethal seedling character, xantha, in a heterozygous condition, but in the work reported here only the homozygous green lines of this stock were used. Iris is a rough-awned, naked-kerneled variety of no commercial importance.

Beside their stripe reactions these varieties had other differentiating morphological characters known to mark particular linkage groups. Thus, crosses have been studied for these characters as well as for stripe reaction. Classifications for these characters were made on F_2 plants and verified in noninoculated F_3 families.

TABLE 1.—*Reactions of parental varieties when inoculated artificially with Helminthosporium gramineum Rabh. (Culture C–1) in field and greenhouse trials at Madison, Wisconsin from 1939 to 1944*[a]

Variety	C.I. No.[b]	Location of trials	Trials	Stripe infected plants[c]		
				Low	High	Weighted average
			No.	*Pct.*	*Pct.*	*Pct.*
Lion 28	923 sel.	Greenhouse	62	6	48	15
		Field	16	5	16	9
Lion 36	923 sel.	Greenhouse	56	2	10	6
		Field	32	5	16	8
Persicum	6531	Greenhouse	104	0	0	0
		Field	21	0	0	0
Brachytic	6572	Greenhouse	54	0	0	0
		Field	76	0	0	0
Colsess IV	5979	Greenhouse	32	56	86	73
		Field	38	67	82	77
Oderbrucker	4666	Greenhouse	212	79	91	84
		Field	107	69	91	78
Iris	998	Greenhouse	23	80	93	81
		Field	5	89	95	91

[a] These reactions are based on tests not included in a previous report on stripe reaction of varieties (12).

[b] C.I. denotes accession number of the Division of Cereal Crops and Diseases (Formerly Office of Cereal Investigations), Bureau of Plant Industry, U. S. Department of Agriculture.

[c] Low and high figures are yearly averages. The weighted average is the sum of the yearly average multiplied by the number of tests in each year, divided by the total number of tests.

Method of Testing Segregates

Hybrid material was tested in the F_3 and to a limited extent in the F_1, F_2, and F_4 generations. Because inoculation did not eliminate all plants of a susceptible variety such as Oderbrucker, tests of F_2 plants could not be expected to give a completely reliable indication of their reactions. Inoculation of the F_3 lines, where a large population and several trials could be used, have therefore, been considered to give a more nearly true picture of the reaction of F_2 plants. Twenty-five or thirty kernels per line were used in each inoculation, and each progeny was tested more than once in attempting to eliminate possible escapes or sampling errors. Oderbrucker and the other parental varieties concerned in any particular trial were used as checks, and included once for every 17 or 18 hybrid lines. A number of tests were made of the F_1 and random samples of the F_2 material. The F_3 lines were taken at random from noninoculated populations except in one instance, and each line was tested at least twice. In general, all the F_3 lines

of a cross were tested at once, but because of limited facilities it was not possible to test each line more than once at a given time. Thus the trials were not strictly replications, and comparisons between them could be made only on the basis of the percentage infection of Oderbrucker and the susceptible parent in the respective trials. F_4 lines were made up by bulking the plants from noninoculated rows of F_3 lines which had been highly susceptible or resistant in other tests. Duplicate inoculations were made on F_4 lines.

The stripe disease has been relatively easy to classify, as in most cases the symptoms on stripe-infected plants were obvious, and plants were either healthy or distinctly stripe-infected. Occasionally, questionable plants appeared, but not frequently enough to have any appreciable effect on the results.

INHERITANCE OF RESISTANCE

Lion × Oderbrucker

As shown in table 1 Lion 36 has been resistant under conditions which produced high amounts of infection in Oderbrucker. The reactions of the F_1 and F_2 generations of Lion 36 × Oderbrucker, with the checks of parent varieties, are shown in figure 1. On the basis of the average infection both generations were susceptible. No significant differences appeared between progenies from reciprocal crosses. Lion 28 has appeared to be somewhat more susceptible than Lion 36. In the F_1 of Lion 28 × Oderbrucker the amount of infection was slightly less than in Lion 36 × Oder-

PARENT OR CROSS AND GENERATION		REPLICATIONS	PLANTS	NUMBER OF TESTS IN PERCENTAGE INFECTION CLASSES:[a] 0	15[b]	35	55	75	95	AVERAGE INFECTION
		NO.	NO.							PER CENT
ODERBRUCKER	P	47	996				—	—	—	85
LION 36	P	27	510	—	—	—				7
LION 28	P	19	356	—	—	—	—	—		20
LION 36 × ODERBRUCKER	F_1	6	81				—	—	—	78
RECIPROCAL	F_1	9	153			—	—	—		57
LION 36 × ODERBRUCKER	F_2	4	79				—	—		66
RECIPROCAL	F_2	8	94			—	—	—	—	66
LION 28 × ODERBRUCKER	F_1	9	143			—	—	—	—	63
RECIPROCAL	F_1	5	82			—	—	—		46
LION 28 × ODERBRUCKER	F_2	44	1154		—	—	—	—		42
ODERBRUCKER[c]	P	1	38						—	97
LION[c]	P	1	109	—						3
LION × ODERBRUCKER[c]	F_2	1	117					—		81

FIG. 1. Lion × Oderbrucker. Distribution of tests of parents and F_1 and F_2 populations in percentage classes according to the stripe infection when artificially inoculated.

[a] Parents and F_1 and F_2 populations were placed in infection classes on the basis of individual tests, while F_3 lines and backcross lines were placed on the basis of the averages of 2, 3, or 4 trials as indicated. The height of the column in each class is proportional to the number of tests or lines which fell in that class.

[b] 10.1 to 20.0 per cent inclusive.

[c] Unpublished data from H. L. Shands, 1932.

brucker, and in the F_2 the amount was distinctly less. This was due in part to a lesser infection in the trial in which the major portion of the tests of the F_2 of Lion 28 × Oderbrucker was run. In an earlier test it was found that the F_2 of Oderbrucker × Leiorrynchum (Lion) was very susceptible, as indicated by the data at the bottom of figure 1. From the reactions of the F_1 and F_2 generations it appeared that the susceptibility of Oderbrucker was at least partially dominant.

One hundred sixty-two F_3 lines taken at random from a noninoculated F_2 population were tested 4 times each for their stripe reaction. An analysis of variance of the F_3 data transformed to $\sin^2$ (because the data were expressed as percentages) indicated that there was some differential reaction

PARENT OR CROSS	LINES	REPLICATIONS	NUMBER OF TESTS OR LINES IN PERCENTAGE INFECTION CLASSES:[a] 0 15 35 55 75 95	AVERAGE INFECTION LINES	ODER-BRUCKER CHECKS	LION CHECKS
	NO.	NO.		PCT.	PCT.	PCT.
ODERBRUCKER P	–	78		–	85	–
LION 36 P	–	66		–	–	8
LION 28 P	–	37		–	–	17
LION 36 × ODERBRUCKER F_3 (NON-INOCULATED F_2)	162	4		31	91	3
LION 36 × ODERBRUCKER F_3 (STRIPE-FREE PLANTS FROM INOCULATED F_2)	36	3		35	80	9
ODERBRUCKER × F_1(LION 28 × ODERBRUCKER)	147	3		59	80	15
LION 28 × F_1(LION 28 × ODERBRUCKER)	101	2		21	86	23

FIG. 2. Lion × Oderbrucker. Distribution of parents, F_3 lines, and backcross lines in percentage classes according to the stripe infection when artificially inoculated.
[a] See footnote for Fig. 1.

in lines between replications, but it did not appear to be serious. The lines were placed arbitrarily in 10 per cent reaction classes on the basis of the average infection for 4 replications. When the transformation was used the standard error within these classes was uniform over the whole range of infection percentages, and the minimum significant differences were only slightly larger than the classes. Similar statistical treatments were given the crosses to be discussed subsequently, and the results were almost identical.

The distribution of parental checks and F_3 lines (from noninoculated F_2) is shown in figure 2. The distribution of the lines was fairly normal, though skewed toward the resistant side, with the mode in the 25 per cent

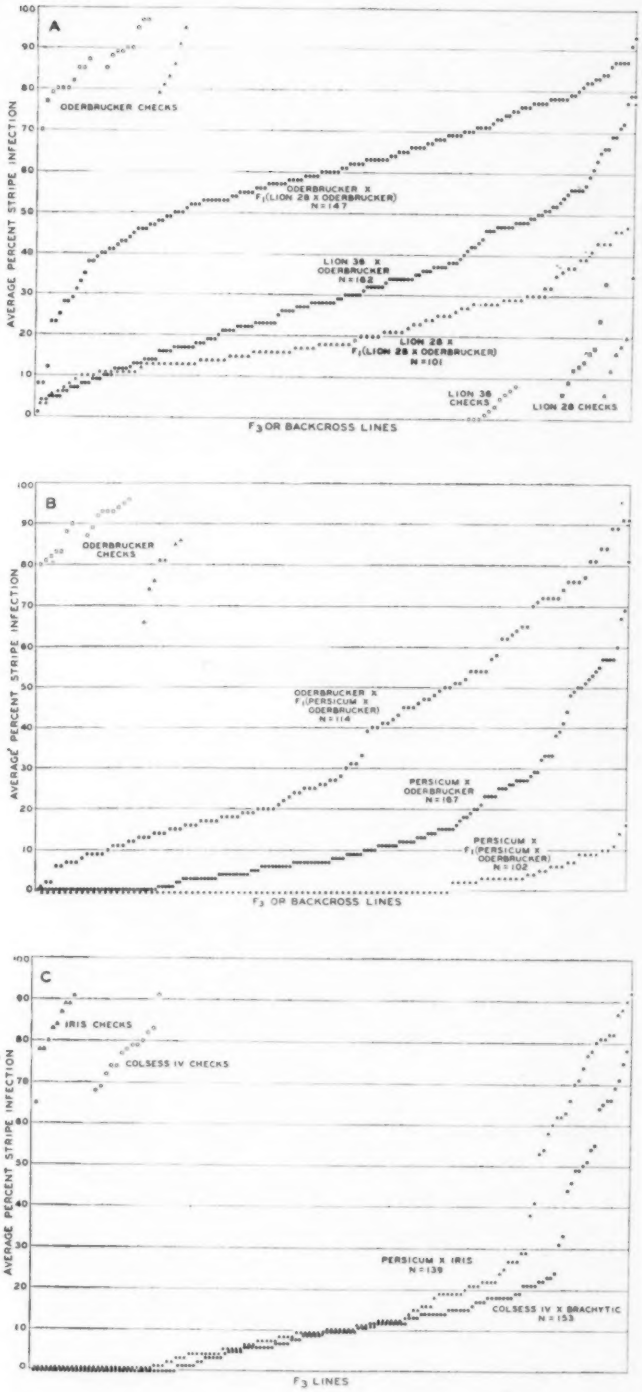

FIG. 3. Distributions of parents, F_3 lines, and backcross lines in order of increasing average stripe infection. Each solid black mark represents a line.

class and the mean in the 35 per cent class of infection. As would be expected from the fact that Lion 36 was not completely resistant there were no F_3 lines in the 0 per cent class. There were few lines in the susceptible end of the distribution, and it was doubtful whether any lines were as susceptible as Oderbrucker. Although the inoculation treatment was rather severe there did not appear to be any marked selective mortality before emergence, since the number of plants per line was nearly as high in the susceptible classes as in the resistant. This is indicated by the following:

Percentage infection classes	5	15	25	35	45	55	65	75
Average number of plants per line (100 kernels planted)	66	68	67	65	64	64	63	69

The distribution of the F_3 lines in order of increasing average stripe infection is shown in figure 3, A. The curve formed showed a continuous variation, without discrete classes of resistance or susceptibility.

Thirty-six lines obtained from F_2 plants which remained stripe-free under a severe inoculation of an F_2 population were tested for stripe reaction. About 65 per cent of the F_2 plants had been eliminated by stripe infection. The distribution of the remaining lines, based on the average of 3 tests each, is given in figure 2 under the designation (Inoculated F_2). The distribution appeared to be very similar to that of the lines from the noninoculated F_2. The data indicated that the F_2 test did little to select for resistant plants.

Part of the lines from the noninoculated F_2 population were tested further in the F_4. These lines were chosen because they were either high or low in amount of infection in the F_3 tests. The F_4 lines were made up by bulking plants from noninoculated F_3 rows. The data are in table 2. The infection averages for the 2 generations agreed very well. In these tests it appeared that lines 21, 84, and 106 were as susceptible as Oderbrucker, and 13 lines were as resistant as Lion 36, if 15 per cent were considered the upper limit for resistance.

F_1 (*Lion 28* × *Oderbrucker*) × *Oderbrucker*

The backcross plants were grown without inoculation, and the lines obtained tested for stripe reaction. Results for reciprocals were combined because of their similarity. The distribution of the lines, as given in figure 2, showed a definite shift toward susceptibility when compared to the simple cross. The average infection in the F_3 lines was increased by 28 per cent by the introduction of another complement of the susceptible genotype. The distribution of the backcross lines in order of increasing average infection is shown in figure 3, A. The curve showed considerably higher infection than that for the simple cross, and there appeared to be no obvious breaks in continuity.

F_1 (*Lion 28* × *Oderbrucker*) × *Lion 28*

The distribution of the lines of this backcross tested for stripe reaction also appear in figure 2. The results for reciprocals were combined.

Eighty-five per cent of the lines seemed to be as resistant as the Lion 28 checks. The 3 lines in the 0 per cent class showed some stripe on further testing. The resistance of Lion 28 appeared to be dominant in this cross. The distribution of the lines in order of increasing average infection is

TABLE 2.—*Comparison of percentage stripe infection of high and low F_3 lines with percentage infection in corresponding F_4 lines made up by bulking uninoculated F_3 rows*

Lion × Oderbrucker			Persicum × Oderbrucker		
Line number	F_3 Average[a]	F_4 Average[b]	Line number	F_3 Average[c]	F_4 Average[c]
	Pct.	*Pct.*		*Pct.*	*Pct.*
65	1	9	27	81	64
121	4	9	60	69	36
177	4	32	97	69	40
81	5	12	34	60	28
165	5	9	72	57	22
166	5	27	100	57	18
167	5	10	110	57	17
50	6	14	28	55	27
93	6	6	7	54	22
14	7	17	166	53	13
83	7	15	57	52	35
186	7	10	43	51	25
18	8	7	Ave.	51	25
43	8	19			
190	8	27	Oderbrucker	93	71
90	9	9	Persicum	0	0
105	9	12			
132	9	21	Brachytic × Oderbrucker		
98	10	6			
117	10	16	Line number	F_3 Average[d]	F_4 Average[e]
Ave.	7	14			
				Pct.	*Pct.*
106	79	71	208	65	45
150	79	62	15	58	37
12	77	48	26	52	37
124	72	62	29	41	26
44	71	48	47	39	6
21	69	71	84	38	45
92	69	54	65	37	33
84	66	76	136	36	39
158	66	63	48	35	46
77	65	66	126	35	30
69	63	65	97	32	58
15	61	33	Ave.	43	37
Ave.	70	60			
Odebrucker	91	75	Oderbrucker	74	60
Lion 36	3	7	Brachytic	0	0

[a] Average of 4 trials, 2 in field and 2 in greenhouse.
[b] Average of 2 trials in greenhouse.
[c] Average of 3 trials.
[d] Average of 4 trials.
[e] Average of 2 trials.

shown graphically in figure 3, A. Again there were no obvious breaks in the curve, and infection percentages were lower than for the simple cross.

The data for the several generations of Lion × Oderbrucker were somewhat contradictory. The amount of infection in the F_1 and F_2 indicated that susceptibility was at least partially dominant; however, the distribution of the F_3 lines indicated that resistance was partially dominant. Tests of selected F_4 lines agreed with the F_3 findings, suggesting that the F_3 classifications were essentially correct, and that the F_3 lines with high and low amounts of infection were relatively pure for stripe reaction. The results with F_3 lines from an inoculated F_2 population suggested that the inoculation of F_2 material had no selective effect towards resistance. If the tests of the F_3 were considered the most reliable, then the skewness of the distribution of lines would indicate that resistance was partially dominant, and the normality of the distribution would suggest that several factors were involved in conditioning stripe reaction. The backcross of the F_1 to Oderbrucker, in which 10 per cent of the lines fell in or above the 85 per cent infection class, suggested that a 3-factor difference was involved.

PARENT OR CROSS AND GENERATION		REPLICATIONS	PLANTS	NUMBER OF TESTS IN PERCENTAGE INFECTION CLASSES: 0 15 35 55 75 95	AVERAGE INFECTION
		NO.	NO.		PER CENT
ODERBRUCKER	P	47	996		85
PERSICUM	P	13	289		0
PERSICUM × ODERBRUCKER	F_1	22	338		1
PERSICUM × ODERBRUCKER	F_2	26	557		6

FIG. 4. Persicum × Oderbrucker. Distribution of parents and F_1 and F_2 populations in percentage infection classes according to the stripe infection when artificially inoculated.

The backcross of the F_1 to Lion seemed to substantiate this possibility by showing 85 per cent of the lines in the 25 per cent infection class or below. The resistance of Lion was apparently dominant in these backcross lines. The ease with which parental types were regained in backcrosses suggested that the inheritance was not complex.

Persicum × Oderbrucker

Persicum has been highly resistant in all inoculation tests made with culture C–1. The reactions of the F_1 and F_2 generations of the cross Persicum × Oderbrucker are given in figure 4. The results for reciprocals were combined. Most of the F_1 plants were resistant when either variety was used as the female parent. The F_2 generation had a slightly higher number of stripe-infected plants. The resistance of Persicum appeared to be dominant.

The distribution of the F_3 lines, based on the average infection, is shown in figure 5. A large proportion of the lines were highly resistant, with 20 per cent of them in the 0 per cent infection class. No lines appeared to be as susceptible as Oderbrucker, although one line in the 85 per cent class

approached it. The distribution of the F_3 lines in order of increasing average infection appears in the middle curve of figure 3, B. The curve formed suggested no definite divisions except the one susceptible line and the highly resistant class. No differential mortality appeared in the higher infection classes as the average number of plants per line in the higher classes was nearly the same as in the lower classes, as shown in the following:

Percentage infection classes	0	5	15	25	35	45	55	65	75	85
Average number of plants per line (75 kernels planted)	68	70	68	68	66	64	63	73	.....	44

There was a slight downward trend from the 5 per cent class to the 55 per cent class. The 65 and 85 per cent classes contained only 2 lines and 1 line, respectively.

PARENT OR CROSS	LINES	REPLICATIONS	NUMBER OF TESTS OR LINES IN PERCENTAGE INFECTION CLASSES:[a] 0 15 35 55 75 95	AVERAGE INFECTION LINES	ODERBRUCKER CHECKS	PERSICUM CHECKS
	NO.	NO.		PCT.	PCT.	PCT.
ODERBRUCKER P	–	62		–	85	–
PERSICUM P	–	62	62	–	–	0
PERSICUM × ODERBRUCKER F_3	167	3	62	15	93	0
ODERBRUCKER × F_1(PERSICUM × ODERBRUCKER)	81	3		41	84	0
RECIPROCAL	33	3		25	84	0
PERSICUM × F_1(PERSICUM × ODERBRUCKER)	102	2	71	2	78	0

FIG. 5. Persicum × Oderbrucker. Distribution of parents, F_3 lines, and backcross lines in percentage infection classes according to the stripe infection when artificially inoculated.

[a] See footnote for Fig. 1.

Lines that were highly resistant or relatively susceptible in the F_3 were tested again in the F_4 as a check on the F_3 classification. These F_4 lines were from bulked, noninoculated F_3 rows. The averages for the 2 generations of these lines are in table 2. The inoculation tests for the F_4 lines were less severe than for the F_3 lines, as is shown by the lower average for the Oderbrucker checks. With the exception of lines 68 and 87 those lines which showed no infection in the F_3 also showed none in the F_4. There were 17 lines with no stripe-infected plants in either the F_3 or the F_4. Lines with high infection in the F_3 had some susceptibility in the F_4, but only line 27 appeared to be as susceptible as Oderbrucker.

F_1 (*Persicum* × *Oderbrucker*) × *Oderbrucker*

F_1 plants of Persicum × Oderbrucker were backcrossed to Oderbrucker, and the crossed seed grown to produce backcross lines. The F_1 plants were

used both as male and female parent. The distribution of the backcross lines is shown in figure 5. The average infection in the lines in which the hybrid was the female was less than in those in which Oderbrucker was the female (21 per cent compared to 41 per cent). The distributions of the progenies from reciprocal crosses were essentially similar, however, and both showed a definite trend towards susceptibility when compared to the distribution for the simple cross. There were 18 lines in the 75 and 85 per cent classes which thus appeared to be as susceptible as the Oderbrucker checks. Since every backcross line had at least half of the Oderbrucker gene complement, and resistance was not completely dominant, no lines would be expected in the 0 per cent class. The distribution of the lines in order of increasing average infection is shown in the upper curve of figure 3, B. Breaks in the distribution were suggested at the 35 and 65 per cent levels.

F_1 (*Persicum* × *Oderbrucker*) × *Persicum*

Similar crosses were made with Persicum as the recurrent parent, and a limited number of lines tested for stripe reaction (Fig. 5). Seventy per cent of the lines appeared to be stripe-free, and the balance showed only a low percentage of infection. Resistance appeared to be partially dominant. The distribution of the lines in order of increasing average infection is shown in the lower curve of figure 3, B.

Based upon the amount of infection in the F_1 population of Persicum × Oderbrucker it may be concluded that the factors responsible for resistance in Persicum were almost completely dominant over the factors for susceptibility in Oderbrucker. This dominance was evident also in the results of the studies of the F_2, F_3, and backcrosses. In the several tests of F_2 populations there was a total of 525 healthy plants and 32 stripe-infected plants. This very closely approximates a 15 to 1 or dihybrid ratio, P lying between 0.95 and 0.50. This would suggest that Persicum differed from Oderbrucker in 2 factor pairs, either one of which gave resistance. This hypothesis does not take into account the escapes in the F_2 tests.

The behavior of the F_3 and backcross lines also suggests a factorial hypothesis for the difference in stripe reaction between Persicum and Oderbrucker. In the distribution of F_3 lines approximately 20 per cent of the lines were as resistant as Persicum, and only 1 line (less than 1 per cent) as susceptible as Oderbrucker. These 2 classes may be considered as homozygous resistant and susceptible respectively, although the resistant class likely contained some heterozygous lines. All the lines between these extremes were probably heterozygous in various degrees and there appeared to be no logical means of separating them further. Although 2 factors were suggested by the F_2 tests, the low number of susceptible lines in the F_3 made it seem likely that at least 1 additional factor was involved.

If it is assumed that Persicum has 3 independent, dominant factor pairs for resistance, and that Oderbrucker has the recessive allelomorphs for

complete susceptibility, the F_2 plants of a cross between these 2 genotypes (AABBCC and aabbcc) might be grouped on the basis of their F_3 breeding behavior as follows: 10/64 would give no segregation in the F_3 for at least 2 dominant factor pairs and would be highly resistant in the F_3, 53/64 in the F_3 would segregate or be homozygous recessive for at least 2 of the factor pairs, and 1/64 would be true-breeding recessive in the F_3 and completely susceptible.

This would give a ratio of 10 highly resistant : 53 intermediate : 1 susceptible. The comparison of the calculated with the observed ratio indicates that the agreement was satisfactory.

	Observed	Calculated	
Highly resistant	34	26.1	$\chi^2 = 3.66$
Intermediate	132	138.3	P lies between 0.2 and 0.1
Susceptible	1	2.6	

On the basis of this hypothesis it would be expected that one-eighth of the backcross lines, with Oderbrucker as the recurrent parent, would be susceptible. If the backcross lines in the 75 and 85 per cent infection classes were considered as susceptible, the agreement of the observed segregation with the expected was good.

	Observed	Calculated	
Intermediate	96	100	$\chi^2 = 1.30$
Susceptible	18	14	P lies between 0.3 and 0.2

In the backcross with Persicum as the recurrent parent about 70 per cent of the lines were highly resistant. This suggested a 2-factor difference between the 2 parents. If this backcross were to fit the 3-factor hypothesis suggested above, 50 per cent of the lines should show some stripe-infected plants. This was obviously not the case, although further tests might bring closer agreement.

Brachytic × *Oderbrucker*

Brachytic has been highly resistant in tests using culture C–1. In crosses with Oderbrucker the F_1 and F_2 generations reacted to stripe as shown in figure 6, A. The reactions were essentially the same as in the cross Persicum × Oderbrucker.

Four stripe tests were made with each F_3 line of this cross. One of these was in the greenhouse and the others were in the field. Because of dry soil the field tests were poor and the infection very low. The distribution of the F_3 lines on the basis of the average of the 4 tests is given in figure 6, A. It was very similar to that for Persicum × Oderbrucker, except that the average infection for all lines was lower. The distribution of the lines in order of increasing average infection was also every similar and thus is not shown here. Again a few lines approached the susceptibility of Oderbrucker. This lack of susceptible lines did not seem to be the result

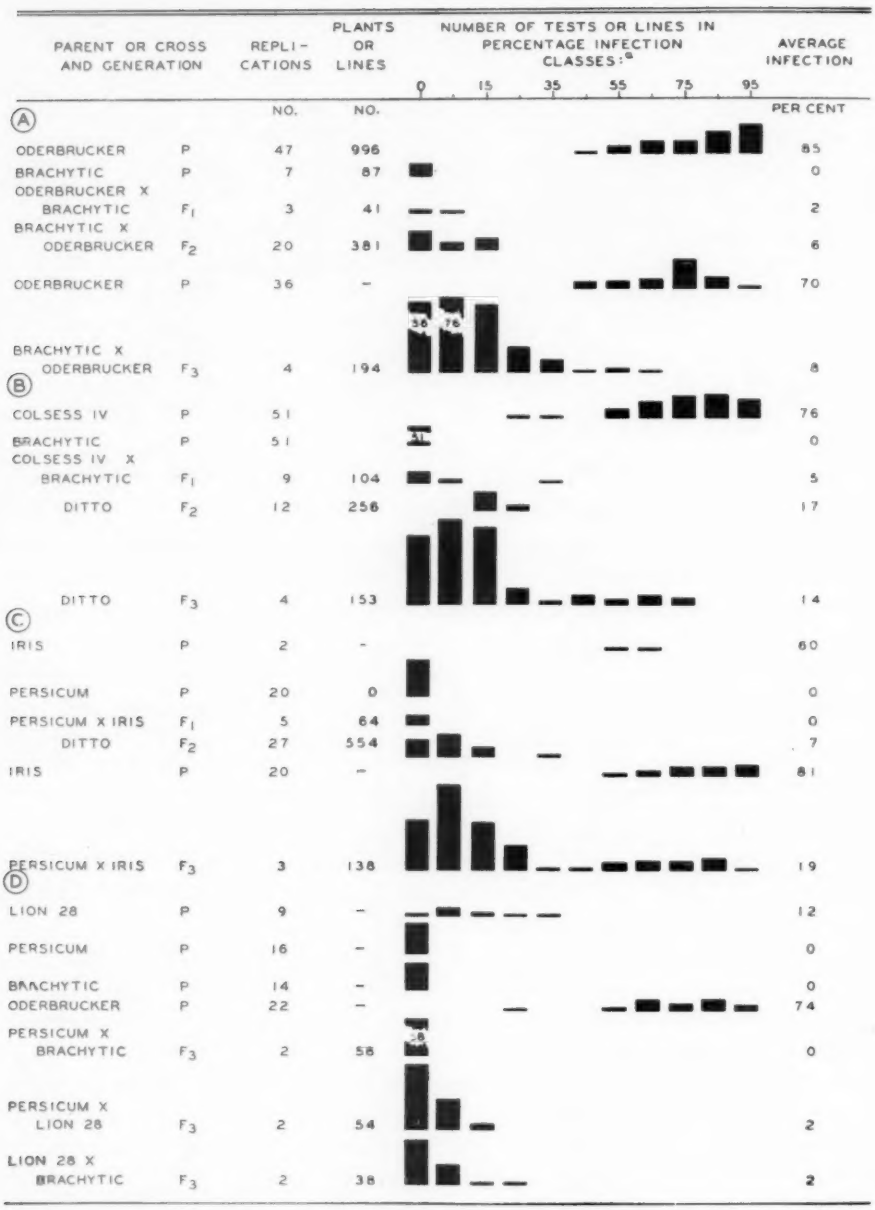
PARENT OR CROSS AND GENERATION
REPLI-CATIONS
PLANTS OR LINES
NUMBER OF TESTS OR LINES IN PERCENTAGE INFECTION CLASSES:[a]
0
15
35
55
75
95
AVERAGE INFECTION
NO.
NO.
PER CENT
(A)
ODERBRUCKER P 47 996 85
BRACHYTIC P 7 87 0
ODERBRUCKER X BRACHYTIC F1 3 41 2
BRACHYTIC X ODERBRUCKER F2 20 381 6
ODERBRUCKER P 36 - 70
BRACHYTIC X ODERBRUCKER F3 4 194 8
(B)
COLSESS IV P 51 76
BRACHYTIC P 51 0
COLSESS IV X BRACHYTIC F1 9 104 5
DITTO F2 12 256 17
DITTO F3 4 153 14
(C)
IRIS P 2 - 60
PERSICUM P 20 0 0
PERSICUM X IRIS F1 5 64 0
DITTO F2 27 554 7
IRIS P 20 - 81
PERSICUM X IRIS F3 3 138 19
(D)
LION 28 P 9 - 12
PERSICUM P 16 - 0
BRACHYTIC P 14 - 0
ODERBRUCKER P 22 - 74
PERSICUM X BRACHYTIC F3 2 58 0
PERSICUM X LION 28 F3 2 54 2
LION 28 X BRACHYTIC F3 2 38 2

FIG. 6. Brachytic × Oderbrucker, Colsess IV × Brachytic, Persicum × Iris, Persicum × Brachytic, Persicum × Lion 28, and Lion 28 × Brachytic. Distribution of parents, F_1 and F_2 populations, and F_3 lines in percentage infection classes according to the stripe infection when artificially inoculated.

[a] See footnote for Fig. 1.

of differential mortality of stripe-infected plants before emergence as shown in the following:

Percentage infection classes	0	5	15	25	35	45	55	65
Average number of plants per line (120 kernels planted)	76	66	67	68	66	84	62	87

There were only 2 and 3 lines in the 55 and 65 per cent classes respectively.

Lines with no infection or with relatively high infection in the F_3 were tested again in the F_4 as bulks from noninoculated F_3 rows. The amount of infection in the Oderbrucker checks in these tests was low. A comparison of the F_3 and F_4 is in table 2. With the exception of lines 58, 114, 118, and 142, lines with no infection in the F_3 had none in the F_4. These exceptions indicated that some escapes occurred in the F_3 tests. There were 19 lines with no stripe-infected plants in either the F_3 or the F_4. Lines that were high in infection in the F_3 tests also had fairly high infection in the F_4.

In general the several generations of the cross Brachytic × Oderbrucker have reacted to stripe inoculation in the same manner as did those of the cross Persicum × Oderbrucker. The presence of 2 dominant factor pairs in Brachytic was suggested by the F_2, as the agreement with the 15 to 1 ratio expected on this basis was satisfactory, P lying between 0.5 and 0.3. There were 362 healthy plants and 19 stripe-infected plants in the several F_2 tests. Because the F_3 tests were not severe, infection in the lines was generally low. Resistance appeared to be dominant over susceptibility, and apparently more than 1 factor difference was involved. The significance of the fact that slightly more than one-fourth of the F_3 lines were highly resistant was problematical. Tests of certain F_4 lines showed that the F_3 classifications were essentially correct, and that some degree of homozygosity for stripe reaction had already been reached within the lines. The 3-factor hypothesis which seemed to fit the Persicum × Oderbrucker F_3 and backcross results, did not give satisfactory agreement for the progeny of Brachytic × Oderbrucker.

Colsess IV × Brachytic

Colsess IV has been highly susceptible to the culture of the stripe fungus used in these tests. The results for Colsess IV × Brachytic are shown in figure 6, B. Inoculation of F_1 plants gave 8 per cent infection, indicating a partial dominance for the Brachytic resistance. In the F_2 generation 212 plants remained healthy and 44 became stripe-infected, a ratio of 4.8 to 1 or an average of 17 per cent infection. The distribution of the F_3 lines was distinctly different from the Lion × Oderbrucker or the Persicum × Oderbrucker type, because in Colsess IV × Brachytic there were relatively more susceptible lines. Twenty-five per cent of the lines had no infection, and 6 per cent had above 60 per cent. If the distribution is divided accordingly the segregation closely approximates that expected on the basis of a difference of 2 factor pairs, 1 major and 1 modifier.

	Observed	Calculated	
4 highly resistant	37	38.2	$\chi^2 = 0.109$
11 intermediate	107	105.2	P lies between 0.95 and 0.5
1 susceptible	9	9.6	

This division at the 60 per cent infection level was indicated by the distribution of the lines in order of increasing average infection as shown in figure 3, C. There was also a rather definite break at the 25 per cent level, but the significance of this was not evident.

Persicum × *Iris*

Iris has been highly susceptible to the fungus culture used in these tests. Iris was crossed with Persicum, and the several generations tested for stripe reaction. The distributions are given in figure 6, C. Only a few plants were tested in the F_1 but they all remained healthy, indicating that resistance was dominant. In the F_2 there were 299 disease-free plants and 19 stripe infected. This closely approximates a 15 to 1 ratio ($\chi^2 = 0.05$; P lies between 0.95 and 0.50).

The distribution of the F_3 lines was very similar to that of the Colsess × Brachytic F_3 progenies. There were 11 lines with 75 per cent or more infection. If these were considered susceptible then the observed and calculated ratios would be as follows:

	Observed	Calculated	
Highly resistant	28	33.6	$\chi^2 = 2.20$
Intermediate	100	92.4	P lies between 0.5 and 0.3
Susceptible	11	8.4	

This again indicated a 2-factor difference with 1 major and 1 modifying factor. However, the break at the 75 per cent level might be questionable, as it was not definitely indicated on the curve in figure 3, C, formed by the distribution of the lines in order of increasing average infection. This curve is strikingly similar to the corresponding one for Colsess IV × Brachytic.

Persicum × *Brachytic*

Both of the varieties in this cross have been highly resistant in tests with culture C–1. F_3 lines of the cross have been tested for their stripe reaction. Figure 6, D, shows that while fairly high amounts of infection occurred in the Oderbrucker checks, no stripe-infected plants appeared in any of the F_3 lines or in either of the parents. This lack of segregation would indicate that there were some factors for resistance common to both Persicum and Brachytic.

Persicum × *Lion 28*

The F_2 of Lion × Persicum gave very low infection on inoculation, with an average of 2.5 per cent in 8 tests involving 198 plants. F_3 lines have also been tested and their distribution is shown in figure 6, D. The average infection for the F_3 lines was very low, but stripe-infected plants

appeared in some lines. In none of the lines did the amount of infection exceed that which occurred in the Lion 28 checks. Because segregation occurred in the F_3 lines of this cross it may be concluded that there was either a quantitative or a qualitative difference in the factor or factors conditioning resistance in the 2 varieties.

Lion 28 × Brachytic

The distribution of the F_3 lines is shown in figure 6, D. This cross was very similar in its behavior to the Persicum × Lion 28 cross and the conclusions drawn were the same.

ASSOCIATION OF CHARACTERS

In addition to the studies of the stripe reactions of the progenies of the various crosses, observations were made concerning the inheritance of certain morphological characters. Classifications were based upon F_2 plants and verified in the F_3 lines, except in the backcrosses, where only the backcross plants were classified. Although the populations were relatively small, attempts were made to verify previous reports as to the mode of inheritance of the genes and their relationships to each other. A summary of linkage studies and a list of references on mode of inheritance has been given by Robertson, Wiebe, and Immer (10), and therefore most of the references will not be cited here. Tests for goodness of fit and independence were made by means of Chi-square. These tests are not reported in detail, but a P value of 0.95 to 0.05 was regarded as indicating satisfactory agreement. The principal object was to determine the relations of these genes to stripe reaction.

Lion × Oderbrucker

Lion 36 has a black lemma and pericarp, smooth awns, long-haired rachillas, practically no hairs on the glumes, and glume awns 7 to 10 mm. long. In contrast Oderbrucker has a white lemma and pericarp, rough awns, short-haired rachillas, glumes with abundant hairs, and glume awns 16 to 25 mm. long.

Long-haired vs. short-haired rachilla and black vs. white lemma and pericarp have been reported to be conditioned by single independent factor pairs. The general classification of rough vs. smooth awns has been found previously to be conditioned by a single factor pair, with at least 1 other pair modifying the degree of smoothness. Neatby (7) has determined that in certain varieties glume length acts as a dihybrid in its inheritance. Hor (3) reported that the factor affecting rachilla hair length also affected the nature of pubescence of the glume in a similar way, while the extent of coverage of the glumes by hairs was governed by another factor pair. Daane (2) found the factors governing rough vs. smooth awns and long- vs. short-haired rachillas to be linked.

In the cross Lion 36 × Oderbrucker the inheritance of rachilla hair

length, glume awn length, lemma color, and rough vs. smooth awns were found to be governed by a single factor pair in each case. Glume pubescence was an unsatisfactory character to classify because all gradations from no hairs through various widths of bands of hairs to complete coverage occurred. At least 3 factor pairs were apparently involved in the inheritance of glume pubescence in this cross. The factors for rough awns and short-haired rachillas appeared to be linked, with a percentage recombination of 26 ± 2.9 when computed according to the method of Immer and Henderson (4). Hairy glumes appeared to be linked wtih both long glume awns and rough awns. Factors for other characters studied seemed to segregate independently of each other. By observation (Table 3) the stripe classes showed no definite association with any of these 5 characters, and independence was indicated by Chi-square tests in all instances.

Lemma color, rachilla hair length, and stripe reaction were studied in the backcross Oderbrucker $\times F_1$ (Lion 28 $\times$ Oderbrucker) and its reciprocal. The reciprocals were considered separately, but there was no evidence of disagreement between them. It was found that the lemma color and rachilla hair length were each monogenic, and independent of each other and of the stripe classes. The average stripe reactions for the several genotypes are in table 3.

Glume awn length, rough vs. smooth awns, glume pubescence, and stripe reaction were studied in the backcross Lion 28 $\times F_1$ (Lion 28 $\times$ Oderbrucker) and its reciprocal. Glume awn length and rough vs. smooth awns were monogenic and independent in their inheritance. Glume pubescence here appeared to be governed by 2 factors and hairy glumes were associated in some way with both long glume awns and rough awns. All 3 morphological characters were independent of stripe reaction. The independence of the characters and stripe reaction was also indicated by the infection averages for the various genotypes in table 3.

Persicum $\times$ *Oderbrucker*

Persicum has a black lemma and pericarp, smooth awns, long-haired rachillas, short glume awns, and infertile lateral florets (2-rowed type). Oderbrucker has fertile lateral florets, as well as the characters described under the previous cross. Segregation for lemma color, rough vs. smooth awns, rachilla hair length, and glume awn length agreed satisfactorily with the assumptions of monogenic inheritance. Varieties with infertile lateral florets have been reported by a number of workers to differ in their genotype from those with fertile lateral florets by a single dominant factor pair. In the present study, the segregation for row number did not fit a 1:2:1 ratio because of an excess of non-2-rowed segregates. F_2 plants were taken at random to make up the F_3 lines, the only restriction being that there be sufficient kernels on each for the F_3 tests. This would tend to favor the non-2-rowed class at the expense of the 2-rowed and heterozygous classes because these latter produce relatively few kernels per plant.

When the remainder of the population from which the lines were taken was classified and the results combined with those for the F_3 lines tested for

TABLE 3.—*Number of lines and average infection for various genotypes and several crosses*

Cross	Homozygous dominant			Heterozygous			Homozygous recessive		
	Genotype[a]	Lines	Ave. stripe infec.	Genotype[a]	Lines	Ave. stripe infec.	Genotype[a]	Lines	Ave. stripe infec.
		No.	*Pct.*		*No.*	*Pct.*		*No.*	*Pct.*
Lion 36 × Oderbrucker	SS	45	33	Ss	85	31	ss	32	31
	E_2E_2	31	30	E_2e_2	89	33	e_2e_2	42	29
	BB	36	37	Bb	83	32	bb	43	26
	RR	38	32	Rr	82	32	rr	42	30
	PdgPdg	101	32	Pdgpdg	57	29	pdgpdg	4	28
Oderbrucker × F_1 (Lion 28 × Oderbrucker)	.....	.....	.....	BB	82	52	bb	82	55
	.....	.....	.....	Ss	82	54	ss	82	53
Lion 28 × F_1 (Lion 28 × Oderbrucker)	.....	.....	.....	Rr	42	20	rr	59	20
	.....	.....	.....	E_2e_2	52	23	e_2e_2	49	17
Persicum × Oderbrucker	VV	36	12	Vv	74	12	vv	57	19
	SS	30	9	Ss	95	16	ss	42	16
	BB	41	14	Bb	83	15	bb	43	15
	RR	41	18	Rr	84	12	rr	42	15
	E_2E_2	48	15	E_2e_2	76	15	e_2e_2	43	13
Oderbrucker × F_1 (Persicum × Oderbrucker)	.....	.....	.....	Vv	68	34	vv	50	29
	.....	.....	.....	Bb	57	38	bb	61	28
	.....	.....	.....	Ss	72	30	ss	46	37
Persicum × F_1 (Persicum × Oderbrucker)	.....	.....	.....	Rr	53	3	rr	49	1
	VV	48	2	Vv	54	2	.....	.....	.....
	.....	.....	.....	E_2e_2	53	2	e_2e_2	49	2
Brachytic × Oderbrucker	BrBr	56	9	Brbr	108	8	brbr	30	11
	SS	53	8	Ss	97	10	ss	44	7
	NN	54	10	Nn	102	9	nn	38	7
Colsess IV × Brachytic	BrBr	49	16	Brbr	83	13	brbr	21	17
	SS	39	17	Ss	77	13	ss	37	14
	NN	48	14	Nn	77	14	nn	28	15
	KK	39	15	Kk	78	14	kk	36	13
Persicum × Iris	BB	16	8	Bb	64	16	bb	44	24
	RR	54	19	Rr	53	13	rr	17	25
	NN	41	12	Nn	55	19	nn	28	21
	VV	26	7	Vv	54	19	vv	44	21
	E_2E_2	42	16	E_2e_2	52	17	e_2e_2	30	20

[a] S—rachilla hairs long, s—rachilla hairs short.
E_2—Glume awns long, e_2—glume awns short.
B—black lemma and pericarp, b—white lemma and pericarp.
R—rough awns, r—smooth awns.
Pdg—glumes hairy, pdg—glumes glabrous.
V—non-six-rowed, v—six-rowed.
Br—plants normal, br—plants brachytic.
N—caryopses hulled, n—caryopses naked.
K—hooded, k—awned.

stripe reaction, the segregation then fit a 1:2:1 ratio satisfactorily for 2-rowed : heterozygous : non-2-rowed.

Tests indicated that rough vs. smooth awns and rachilla hair length were associated, with a percentage recombination of 28 ± 3.3. The independence of lemma color and glume awn length, and row number and glume awn length was questionable. The factors that governed the other characters in Persicum × Oderbrucker were independent. Also there did not appear to be any definite association between the stripe classes and any one of the 5 morphological characters, either according to the Chi-square tests or by observation (Table 3).

For the backcross Oderbrucker × F_1 (Persicum × Oderbrucker) and its reciprocal, there was some lack of agreement between reciprocals, due perhaps to the small number of lines. The segregation for lemma color indicated monogenic inheritance. For rachilla hair length and row number the segregations of reciprocals did not quite agree, but single factors were suggested. Row number and lemma color were independent of each other. With Oderbrucker as the female, row number and lemma color both appeared to be independent of rachilla hair length, but when the F_1 was the female they did not. According to Chi-square tests each of these 3 characters was independent of the stripe-reaction classes. The averages for the various genotypes substantiate this evidence (Table 3).

For the backcross Persicum × F_1 (Persicum × Oderbrucker) and its reciprocal, rough vs. smooth awns, long- vs. short-glume awns, and 2-rowed vs. non-2-rowed were indicated as monogenic, and independent of each other and of the stripe classes. The data in table 3 also suggest this independence.

In table 3 there are some trends of average infection that may represent associations, *e.g.*, in Persicum × Oderbrucker and in F_1 (Persicum × Oderbrucker) × Oderbrucker vv was higher in infection than VV or Vv, but these trends do not seem to be consistent enough to be considered significant. In Oderbrucker × F_1 (Persicum × Oderbrucker) and its reciprocal the heterozygous black lines had higher infection than the homozygous white lines, but this is the opposite of what would be expected if an association were involved.

Brachytic × Oderbrucker

In Brachytic the caryopses are naked when threshed, rachilla hairs are long, and the plants have much shortened internodes. In Oderbrucker the lemma and palea adhere tightly to the caryopses, rachilla hairs are short, and the plants are normal in height.

The brachytic character has been found by Powers (8) and Swenson (15) to be conditioned by a single factor pair. Hulled vs. naked has been reported as a monogenic character by several workers. In the cross Brachytic × Oderbrucker the segregation for brachytic did not fit the expected 1:2:1 ratio satisfactorily because of a deficiency in the brachytic class. Since brachytic plants in general produced fewer kernels than did normal plants there was probably selection against the brachytic character

in picking lines with sufficient seed for the F_3 tests. However, there were enough brachytic lines included in the tests to show any association with stripe resistance if it were definitely present. Rachilla hair length and hull adherence each appeared to be monogenic in their inheritance. Normal vs. brachytic, long- vs. short-haired rachillas, and hulled vs. naked were independent of each other and of stripe-reaction classes. The independence of genotype and stripe classes is indicated by the averages in table 3.

Colsess IV × Brachytic

In Colsess the hulls are adherent, there are hoods instead of awns, rachilla hairs are short, and the plants are normal in height. Hooded vs. awned has been reported as being conditioned by 1 factor pair.

TABLE 4.—*Distribution of F_3 lines of Colsess IV × Brachytic for reaction to stripe and covered smut.*[a] *Lines placed according to average of 4 tests for each disease*

Covered smut	Stripe									
Lines in percentage infection classes[b]	Lines in percentage infection classes[b]									Total
	0	5	15	25	35	45	55	65	75	
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
0	9	15	12	2	1	0	0	1	1	41
5	18	17	12	2	0	1	1	4	1	56
15	4	5	11	4	0	2	2	0	2	30
25	1	4	3	1	0	1	0	0	0	10
35	1	1	1	0	1	1	0	0	0	5
45	2	1	1	0	0	0	0	0	0	4
55	0	1	1	0	0	0	0	0	0	2
65	0	0	0	0	0	0	0	0	0	0
75	1	1	1	0	0	0	0	0	0	3
85	1	1	0	0	0	0	0	0	0	2
Total	37	46	42	9	2	5	3	5	4	153

[a] Covered smut reactions supplied by R. G. Shands.
[b] Each line was tested 4 times for each disease, and placed on the basis of the average of the 4 tests.

For the cross Colsess IV × Brachytic segregations for hooded vs. awned and long- vs. short-haired rachillas each fit the single factor assumptions, while hulled vs. naked and normal vs. brachytic did not. The factors governing the inheritance of these 4 characters were independent of each other and of the stripe classes. The independence of these characters and stripe reaction is also indicated by the averages in table 3.

The lines of this cross had been previously tested for reaction to covered smut (*Ustilago hordei* (Pers.) K. & S.) by R. G. Shands. The distribution for reaction to stripe and smut appears in table 4. For the diseases individually the distributions were very similar. There were no lines highly susceptible to both diseases. There was no correlation between the reactions to the 2 diseases.

Persicum × *Iris*

Iris has a white lemma and pericarp, rough awns, naked caryopses, long glume awns, and is 6-rowed. Persicum carried the alleles for the factors governing the inheritance of each of these characters.

Hulled vs. naked and long vs. short awns each gave satisfactory agreement with 1:2:1 segregation expected on the basis of single factor differences. Segregations for black vs. white, rough vs. smooth awns, and 2-rowed vs. non-2-rowed did not fit those expected for monogenic inheritance. These 5 characters were independent of each other, except hulled and 2-rowed. They were all independent of stripe-reaction classes. The average stripe infections for the various genotypes are in table 3. These seem to suggest some association between stripe resistance and black lemma color, and between stripe resistance and 2-rowed, even though the Chi-square tests did not detect them.

DISCUSSION

The data on the inheritance of stripe reaction have shown that there were genetic differences between varieties which appeared to be similar in their reaction to the disease. In varietal tests Oderbrucker has appeared to be as susceptible as Colsess and Iris, but in crosses with resistant varieties the susceptibility of Oderbrucker was recovered with less frequency than that of Colsess or Iris. The distributions of F_3 lines in order of increasing average infection were rather distinctly different for Persicum × Oderbrucker and Persicum × Iris as shown in figure 3, in that there was a definite break in direction in the Persicum × Iris distribution at the 25 per cent level which was not present in the Persicum × Oderbrucker distribution. Since the resistant parent was the same in both crosses there must have been a genetic difference between Oderbrucker and Iris. Persicum and Brachytic have been shown to have some factors in common for resistance. On the basis of the similarity of the distributions of Colsess × Brachytic and Persicum × Iris it might be concluded that Colsess and Iris have some factors for susceptibility in common.

Attempts to find associations between stripe reaction and certain morphological characters have not been successful. Marker genes in at least two linkage groups were involved in each cross, and among the various crosses marker genes in six of the seven linkage groups of barley, number VI excepted, have been tested for their relationship to stripe reaction (Table 5). However, since the resistances were genetically different and apparently the susceptibilities also, independence in one cross does not justify the conclusion of independence in another cross where that particular marker gene could not be studied. A cross involving marker genes in all linkage groups, as well as differences in stripe reaction, would be helpful in this respect.

The factors conditioning stripe reaction may have been located in other linkage groups than those investigated in any particular cross. Since a

number of factors seemed to be involved it might be more logical to conclude that they were located on several chromosomes, and that they all had approximately equal effects in conditioning resistance. It is also possible that the genes affecting stripe reaction were so far removed from the marker genes on the chromosome that the linkage did not become evident with the methods employed.

TABLE 5.—*Summary of genes and linkage groups studied in various crosses with reference to their stripe reaction*

Linkage group	Lion × Oderbrucker	Persicum × Oderbrucker	Brachytic × Oderbrucker	Colsess IV × Brachytic	Persicum × Iris
I	.....	Vv	.....	.....	Vv
II	Bb	Bb	.....	.....	Bb
III	.....	.....	Nn	Nn	Nn
IV	.....	.....	.....	Kk	.....
V	Rr Ss	Rr Ss	.....	.....	Rr
VI	.....	.....	.....	.....	.....
VII	.....	.....	Br br	Br br	.....

[a] A key to the symbols will be found in the footnote of table 3.

SUMMARY

Two types of resistance and two types of susceptibility to the stripe disease were apparent in the varieties considered. The differences in type were evident from the varietal reactions and from the effects of the differences on the progenies of crosses. Persicum and Brachytic remained highly resistant to the culture of the fungus used. The modes of inheritance indicated that these two varieties had some factors for resistance in common. In crosses with Oderbrucker, resistance appeared to be dominant and three factors were probably involved. The resistance of Lion was not complete, and was apparently inherited in a manner different from that of Persicum. Dominance was not definite and a number of factors seemed to be involved. The susceptibility of Oderbrucker was probably different from that of Colsess and Iris, as in the crosses Persicum × Iris and Colsess × Brachytic a difference of one major and one modifying factor pair was involved in each case.

Marker genes in six of the seven linkage groups present in barley were tested for their relationship to stripe reaction, but no associations were found.

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STUDIES ON CRANBERRY FALSE BLOSSOM

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INTRODUCTION

During the Christmas Meetings of the American Association for the Advancement of Science at Philadelphia, in 1940, the writer exhibited periwinkle (*Vinca rosea* L.) plants that had been cured of aster yellows (*Chlorogenus callistephi* var. *vulgaris* Holmes) by heat treatments (14). Dr. Neil E. Stevens visited the exhibit and suggested that similar treatments should be tried on cranberry plants (*Vaccinium macrocarpon* Ait.) affected by false blossom (*Chlorogenus vaccinii* Holmes). Later he kindly offered to supply diseased cranberries for this purpose. His offer was accepted and on June 11, 1941, about sixty false-blossom plants were delivered to The Rockefeller Institute for Medical Research at Princeton, New Jersey, by Dr. Stevens' son, Mr. Russell B. Stevens. My indebtedness to both is gratefully acknowledged.

Preliminary experiments directed toward a determination of ability of false-blossom plants to endure heat were undertaken immediately. However, cranberries were not well suited for heat-treatment tests. Most of the plants that survived the treatments did not resume growth for several months. A few that started growing soon after treatment produced new foliage slowly and did not blossom. Since foliage symptoms of this disease are not very distinctive it was impossible to say whether any of the treated plants had been cured. It was realized that considerable time and a rather large number of plants would be required to make an adequate study of any effects that heat might have on false blossom in cranberry. It was believed that a preliminary study of thermal relationships of the virus in a host that might prove more suitable than cranberry would be advisable. Therefore an attempt was made to transmit the virus to herbaceous plants.

At the time the work was under contemplation the only known vector of false blossom was the cranberry leafhopper *Euscelis striatulus* (Fallen) (5). As this insect was not readily available and as nothing was known regarding its ability to feed on periwinkle or other herbaceous plants, it was decided to try transmission by dodder, *Cuscuta campestris* Yuncker (1, 10, 11), which had been shown to transmit several other viruses. It was soon found that dodder would transmit false blossom to periwinkle. This permitted investigation of effects of heat on the disease in a plant that was more suitable for experimental purposes than cranberry. It also made available a means of studying other phases of the false blossom problem. Some of the work already has been reported briefly (15, 16, 17, 19). It will be presented in detail in this paper.

MATERIALS AND METHODS

The source of the virus used in all experiments was the diseased cranberry plants received from Dr. Stevens. All healthy cranberry plants needed in the work were grown from seeds obtained from fruits purchased in grocery stores in Princeton, New Jersey. Periwinkle plants were grown from cuttings taken from plants belonging to a clone. The dodder used was from seeds obtained from a virus-free plant of *Cuscuta campestris* growing on a healthy sugar-beet plant. Other species were obtained from seed purchased from commercial seed companies.

All plants were grown in porous clay pots set on benches in greenhouses. The houses were fumigated at weekly intervals to control insects. Cranberries were grown in a mixture of equal parts of sand and peat, other plants in rich garden earth. Heat treatments were given in a room that was described previously (13).

Virus-free dodder was kept growing on healthy tomato or sugar beet plants at all times. When it was needed for experiments, branches from four to six inches long were broken off and placed on plants to be parasitized. Usually only one dodder branch was used in making a transfer. The tip of a dodder branch was placed in the tip of the new host. The dodder usually entwined a stem or leaf petiole within 24 hours, but occasionally it failed to take hold and fell out of the new plant. When this happened it always was put back on the plant from which it had fallen.

TRANSMISSION OF FALSE BLOSSOM BY DODDER

Transmission from cranberry to tomato and periwinkle.—In experiments during the autumn of 1940 and during 1941 false-blossom virus was transmitted from cranberries to several hundred tomato and periwinkle plants. There was at the time some uncertainty as to whether the virus being transmitted was that of false blossom; the possibility that another virus might be present in diseased plants had to be considered. Some evidence against that view was obtained from experiments showing that the virus taken to tomato and periwinkle could invariably be gotten from false-blossom plants. All question as to identity was resolved when the virus was taken from tomato and periwinkle to healthy cranberries and shown to produce typical false-blossom symptoms. A few transmission experiments will be described in detail.

Virus-free dodder was transferred from a healthy tomato plant to 8 false-blossom cranberry plants and to 8 healthy cranberry plants. It was allowed to parasitize the cranberry plants for six weeks. A small branch was then transferred from each cranberry plant to a healthy tomato plant. The dodder was allowed to parasitize the tomato plants for six weeks when it was removed and destroyed. Five of the 8 tomato plants exposed to dodder from false-blossom plants came down with false blossom. The other 3 plants and the 8 plants exposed to dodder from healthy cranberry plants remained healthy in appearance for the 9 months that they were

under observation. The experiment showed that dodder transmitted virus from false-blossom cranberry plants to tomatoes.

Several branches of dodder from false-blossom cranberry plants were placed on each of 6 healthy young plants of *Vinca rosea*. Similar branches from healthy plants were placed on 6 other plants of *Vinca rosea* that were to serve as controls. Two weeks later all of the dodder was removed and destroyed. Sixteen days thereafter clearing of veins appeared in leaves near the tips of two branches on one plant of *Vinca rosea* that had been parasitized by dodder from the false-blossom plants. Soon other false-blossom symptoms appeared in these and other branches of the plant. During the next 2 weeks 4 of the 5 other plants of *Vinca rosea* that had been exposed to false-blossom dodder came down with false blossom. The other plant did not show symptoms of disease until 87 days after it had been exposed to viruliferous dodder. The 6 control plants remained healthy. The experiment showed that virus could be transmitted from false-blossom cranberry plants to *Vinca rosea* by dodder.

In another experiment virus-free dodder was transferred to 30 false-blossom cranberry plants and to 30 healthy cranberry plants. It was allowed to parasitize the plants for three months. Then small branches were transferred from the 60 cranberry plants to 60 sets of healthy plants consisting of one tomato and one periwinkle each. In most instances the branches quickly established unions with the new hosts and grew rapidly. In all cases in which this did not occur more branches from the same sources subsequently were placed on the plants. After the dodder had parasitized the tomato and periwinkle plants for about one month it was removed and destroyed. Of the 30 tomato and 30 periwinkle plants exposed to dodder from healthy cranberry plants all remained healthy up to the time the experiment was ended. Of the 30 tomato plants exposed to dodder from false-blossom cranberry plants all but 6 were diseased, and of the 30 periwinkles exposed to dodder from false-blossom cranberry plants all but 9 were diseased within 32 days after the dodder was removed. The 6 healthy-appearing tomatoes and the 9 healthy-appearing periwinkles were then cut back, as pollarding had been found to hasten the appearance of symptoms. Within 7 weeks after this was done all of the plants were diseased. The experiment showed that virus could be transmitted readily from false-blossom cranberry plants to tomatoes and periwinkles.

Transmission from tomato to tomato.—Virus-free dodder was placed on a healthy and a false-blossom tomato plant. After the dodder had parasitized the plants for 3 weeks small branches from the diseased tomato were transferred to each of 13 healthy young tomato plants. Branches of dodder from the healthy tomato were transferred to each of 3 other healthy young tomato plants. The dodder was left on the plants for about 2 months, when it was removed and destroyed. In due course all of the 13 plants exposed to dodder from the diseased tomato became diseased. The control plants exposed to dodder from the healthy tomato remained healthy.

The experiment showed that false-blossom virus could be transferred readily from tomato to tomato by dodder.

Transmission from tomato and periwinkle to cranberry.—Virus-free dodder was placed on a healthy and on a false-blossom tomato plant. When it had parasitized the plants for about 6 weeks small branches from the diseased tomato were placed on each of 8 healthy cranberry plants. Branches from the healthy tomato were placed on each of 8 other healthy cranberry plants. After the dodder had parasitized the cranberry plants for 33 days it was removed and destroyed. Forty days later the cranberry plants were examined and no symptoms of disease were found. It was realized that symptoms might be slow to appear in the cranberry. Hence it was decided to determine whether or not virus could be obtained from any of the plants.

Virus-free dodder was placed on each of the 16 cranberry plants. After it had parasitized the plants for 6 days small branches were transferred to 16 healthy young tomato plants. After the dodder had grown on the tomato plants for 14 days it was removed and destroyed. Twenty-six days later early symptoms of false blossom appeared in one of the tomato plants that had been exposed to dodder from a cranberry that had been parasitized by false-blossom dodder. Eventually 6 of the 8 tomato plants thus exposed became diseased. The other 2 tomato plants and the 8 tomato plants exposed to dodder from the control cranberry plants remained healthy. The test showed that virus could be obtained from 6 of the 8 cranberry plants that had been parasitized by dodder from diseased tomato plants. About 6 months after this test was made the 6 cranberry plants from which virus was obtained had well-marked symptoms of false blossom. During the following year 4 of the 6 diseased cranberry plants produced flowers with typical false-blossom symptoms; the other 2 diseased cranberry plants did not flower. The experiment proved that false-blossom virus can be transmitted from tomato to cranberry by dodder.

Virus-free dodder was placed on 5 false-blossom and on 5 healthy periwinkle plants. When the dodder had parasitized the periwinkle plants for 3 months it was transferred to 10 healthy cranberry plants. After growing on these plants for 3 months it was removed and destroyed. During the following year all of the 5 cranberry plants on which dodder from diseased periwinkles had been placed had well-marked foliage symptoms of false blossom; one of the plants produced flowers and fruits with typical symptoms of false blossom. The 5 cranberry plants on which dodder from healthy periwinkles was placed remained healthy. The experiment showed that false-blossom virus can be transmitted from periwinkles to cranberries by dodder.

Transmission to other species.—As the work on false blossom was undertaken for the purpose of investigating any beneficial effects that heat might have on diseased plants, no special effort was made to transmit the virus widely. However, attempts were made to take it to such species as were

available at the time it was being transmitted to *Vinca rosea*. The plants that took the disease when exposed to virus-bearing dodder are listed:

Dianthus sp., var. Crown of Perfection	in the Caryophyllaceae
Eschscholzia californica Cham.	Papaveraceae
Pastinaca sativa L., Parsnip	Umbelliferae
Apium graveolens L., Celery, var. Silver self blanching	do
Petroselinum hortense Hoffm., Parsley	do
Daucus carota L., Cultivated carrot	do
Vaccinium macrocarpon Ait., Cranberry	Ericaceae
Vinca rosea L., Periwinkle	Apocynaceae
Phlox Drummondii Hook.	Polemoniaceae
Petunia hybrida Hort.	Solanaceae
Nierembergia frutescens Dur.	do
Schizanthus sp.	do
Salpiglossis sp.	do
Lycopersicon esculentum Mill., Tomato	do
Nicotiana tabacum L., Turkish tobacco	do
Nicotiana glutinosa L.	do
Nicotiana langsdorffii Schrank	do
Nicotiana rustica L.	do
Solanum tuberosum L., Potato	do
Veronica peregrina L.	Scrophulariaceae
Scabiosa atropurpurea L.	Dipsaceae
Dimorphotheca aurantiacum DC, African daisy	Compositae
Brachycome iberidifolia Benth., Swan River daisy	do
Centaurea imperialis Hort.	do
Gaillardia aristata Pursh	do
Tagetes erecta L., African marigold	do
Calendula officinalis L.	do
Tragopogon porrifolius L., Salsify, var. Sandwich Island	do

Some plants that did not take the disease when exposed to virus-bearing dodder are the following:

Begonia sp.	in the Begoniaceae
Prunus persica Stokes, Peach	Rosaceae
Medicago sativa L., Alfalfa	Leguminosae
Callistephus chinensis Nees, China aster	Compositae

The disease was taken to 28 species belonging in 10 different families of plants. The families represented are not closely related. The lowest plant in our scheme of classification is in the pink family and the highest in the Compositae. The species that failed to become diseased were exposed

to virus-bearing dodder for at least 3 months and several alfalfa plants were exposed for more than a year. In each case virus-bearing dodder was replaced by virus-free dodder that was allowed to parasitize the plants for 3 months. At the end of this period the dodder was shown to have remained virus-free. It is presumed that alfalfa and the other 3 plants that did not take the disease are immune.

TRANSMISSION OF FALSE BLOSSOM BY GRAFTING

Cranberry vines are too thin to be grafted readily; it probably is because of this that experimental transmission of false blossom by grafting has not been reported previously. Bergman and Truran (2) observed an apparent case of cranberry false-blossom virus transmission through a natural graft. The writer attempted to transmit the virus in cranberry by grafting diseased scions onto healthy plants, but did not succeed, undoubtedly because the scions did not live long enough to form unions with the stocks. During the past three years false blossom has been passed in series in *Vinca rosea* and in a number of different solanaceous plants by grafting, the whip graft being employed. The plants from which and to which the virus was taken by grafting are the following: from *Vinca rosea* to *Vinca rosea;* from tomato to tomato, Turkish tobacco, and *Nicotiana rustica;* from Turkish tobacco, potato, and *Nicotiana glutinosa* to tomato; from *Nicotiana rustica* to *Nicotiana rustica* and *Nicotiana glutinosa;* from potato to potato; and, from *Nicotiana glutinosa* to *Nicotiana glutinosa.* The minimum period recorded for first appearance of false-blossom symptoms after grafting a plant was 23 days in an experiment in which diseased tomato scions were grafted to healthy tomato plants. The usual incubation period in graft transmissions was about 40 days.

MULTIPLICATION OF VIRUS IN DODDER

Infective dodder retained the virus and remained infective for several months even on plants that were immune from the disease. Viruliferous dodder that was allowed to grow on aster, Begonia, and alfalfa for 3 months or longer retained its ability to infect tomato and carrot, although the aster, Begonia, and alfalfa plants remained healthy in appearance, and virus-free dodder allowed to parasitize them did not become infected. This showed that the plants were not symptomless carriers and suggested that the virus might multiply in the dodder. By using small branches in making successive transfers of infective dodder on alfalfa plants Costa (3) showed that infectivity was retained beyond a point where it should have been lost through dilution by growth if there had been no multiplication. From this evidence he concluded that the virus multiplies in dodder. Similar experiments carried out by the writer confirmed this view. In the most thoroughgoing test that was made dodder was passed successively to 5 healthy tomato and 5 healthy alfalfa plants during a period of about 2 years. In each instance a branch of dodder approximately four inches long

was used in making a transfer. The test was started on October 27, 1942, when a branch of dodder produced on a false-blossom cranberry plant was placed on tomato plant number 1. After the parasite had made good growth on tomato number 1 a branch was used to transfer it to tomato number 2 on November 17, 1942; similarly, a branch was transferred from tomato number 2 to tomato number 3 on December 8, from tomato number 3 to tomato number 4 on December 29, and from tomato number 4 to tomato number 5 on January 16, 1943. Successive transfers were at intervals of about 3 weeks. It was believed that the dodder would not be able to obtain virus from the tomato plants within so short a period after their exposure to infection and that if the parasite remained infected it would mean either that the virus multiplied in it or had not been sufficiently diluted by growth to prevent transmission. During the time the dodder was being transferred from tomato to tomato evidence that alfalfa was immune to infection by false blossom became available. Hence alfalfa was substituted for tomato when the 6th successive transfer was made on February 6, 1943. Alfalfa plants also were used when the 7th, 8th, 9th, and 10th successive transfers were made on April 6 and May 10, 1943, and on April 15 and July 13, 1944, respectively. The periods between transfers to alfalfa plants were about 2, 1, 11, and 3 months, respectively. Each time a transfer was made to a new tomato or alfalfa plant transfers also were made to healthy carrot plants in order to determine whether the dodder had remained infective. It was shown to be infective at the time of each transfer. In the final test dodder from alfalfa plant number 5, on which it had been growing for almost 3 months, was transferred to 10 carrot plants on November 10, 1944. On January 26, 1945, all of the 10 carrots had well-marked symptoms of false blossom. The virus was retained over the entire period and there was no indication that any decrease in the infectivity of the dodder occurred.

It was estimated that on tomato the dodder increased in size, at least, tenfold between each transfer. Thus after parasitizing the 5th tomato plant the virus should have been diluted by growth to 10^{-5}, at least, in case there had been no multiplication in the dodder. The dodder increased more than tenfold on each of the 5 alfalfa plants. Its tendency to overgrow the plants was held in check by frequent and rather severe prunings. If the dodder increased only tenfold per month during the 20 months it was grown on the alfalfa plants the virus should have been diluted to 10^{-20} of the concentration obtaining in the branch taken from the 5th tomato plant. Since the virus in that branch already had been diluted to 10^{-5} of the concentration in the branch from the diseased cranberry, the virus in the branches used in the final test should have contained not more than 10^{-25} of the concentration in the original branch if there had been no multiplication. It seemed unlikely that virus at such a high dilution would give infection even through a dodder union. Hence it was concluded that false-blossom virus multiplied in the dodder. This conclusion was reached with

some reluctance because there was no evidence that on healthy plants infective dodder grew less vigorously than virus-free dodder. Moreover, viruliferous dodder bore normal flowers and viable seeds, and showed no symptoms of disease at any time.

SYMPTOMS

The symptoms of false blossom in cranberry are well known. Some of those observed in tomato, periwinkle, *Calendula*, and *Nicotiana glutinosa* have been described and illustrated elsewhere (16, 17). Hence it seems unnecessary to describe the symptoms produced in the other species to which it was taken. In many plants, including tomato, the virus produced characteristic symptoms in the reproductive organs. It caused virescence, distortion, and gigantism in some or all of the flowering parts. The virus of aster yellows in the China aster and the viruses of several other diseases in the yellows group sometimes cause enlargement of flowers of host plants, but no other virus with which the writer is acquainted produces flower symptoms comparable with those of false blossom. Enlargement and fusion of sepals in flowers borne by false-blossom tomato plants produced the structures characteristic of the disease known as big bud. This, of course, does not prove that false blossom and big bud are closely related. Flower symptoms were not observed in celery, parsley, parsnip, and salsify because the plants used were not old enough to bear flowers at the time they became diseased. Flower symptoms were observed in all other plants to which the virus was transmitted. In some species it caused much less stimulation of flowering and fruiting organs than in others; for example, infected flowers in *Nicotiana rustica* were not greatly enlarged (Fig. 1). The flowering branch shown in the upper row at the left is healthy; all others are diseased and show different degrees of distortion. All species bore flowers that were smaller than normal after they had been diseased for some time. It should be clearly understood that big-bud symptoms in any host of false blossom are onset symptoms, or at least symptoms that appear only during the time the infection is becoming established. In late stages of disease the plants usually bore no flowers whatever. The virus caused all plants to become chlorotic, to assume a more upright habit of growth than is normal, and to produce large numbers of secondary shoots. In general it may be said that false blossom is a yellows type disease that causes marked and specific effects on flowering and fruiting organs.

STRAINS OF FALSE BLOSSOM VIRUS

False-blossom cranberry plants received from Dr. Stevens showed different degrees of severity; some were stunted much more than others. Leaves on badly stunted plants usually were smaller than leaves on plants that had made better growth. At first it was presumed that the severely stunted plants had been diseased for a longer time than larger plants. However, when virus was transmitted from cranberry plants showing dif-

ferent degrees of stunting to periwinkle and tomato plants it was observed that in the latter also the disease showed different degrees of severity. Virus transmittd from badly stunted cranberry plants produced severe stunting in periwinkle and tomato, whereas virus transmitted from less seriously affected cranberry plants produced less stunting in periwinkle and tomato. When tomato plants affected by the viruses from several different cranberry plants were arranged in a row according to severity of stunting they presented a graded series of symptoms. This observation suggested that strains of one virus, rather than several different viruses, were causing the different types of disease. Two of the strains were trans-

FIG. 1. Flowers and fruiting capsules of *Nicotiana rustica* affected by false blossom. Branch in upper row at left is healthy; all others are diseased. The picture shows malformations caused by false blossom in this host. (Photograph by J. A. Carlile.)

mitted in series in periwinkle by grafting. In four successive transfers each strain remained unchanged as judged by the symptoms produced (Fig. 2, A). However, on a plant that was kept under observation for about six months and was affected by the milder of the two strains a branch developed that showed severe symptoms. No branches on the plants affected by the severe strain developed mild symptoms. This aspect of the cranberry false-blossom disease problem deserves further study.

FALSE BLOSSOM NOT TRANSMITTED BY MEANS OF JUICE

It is a well-known fact that false blossom cannot be transmitted to cranberry plants by means of juice inoculations. Nevertheless the possibility existed that it might be transmissible to other species by this means. When it was found that the disease would go to solanaceous plants, at-

tempts were made to transmit it to two host species by the rubbing method of inoculation. Since all attempts to pass the virus in this way were unsuccessful only one experiment will be described.

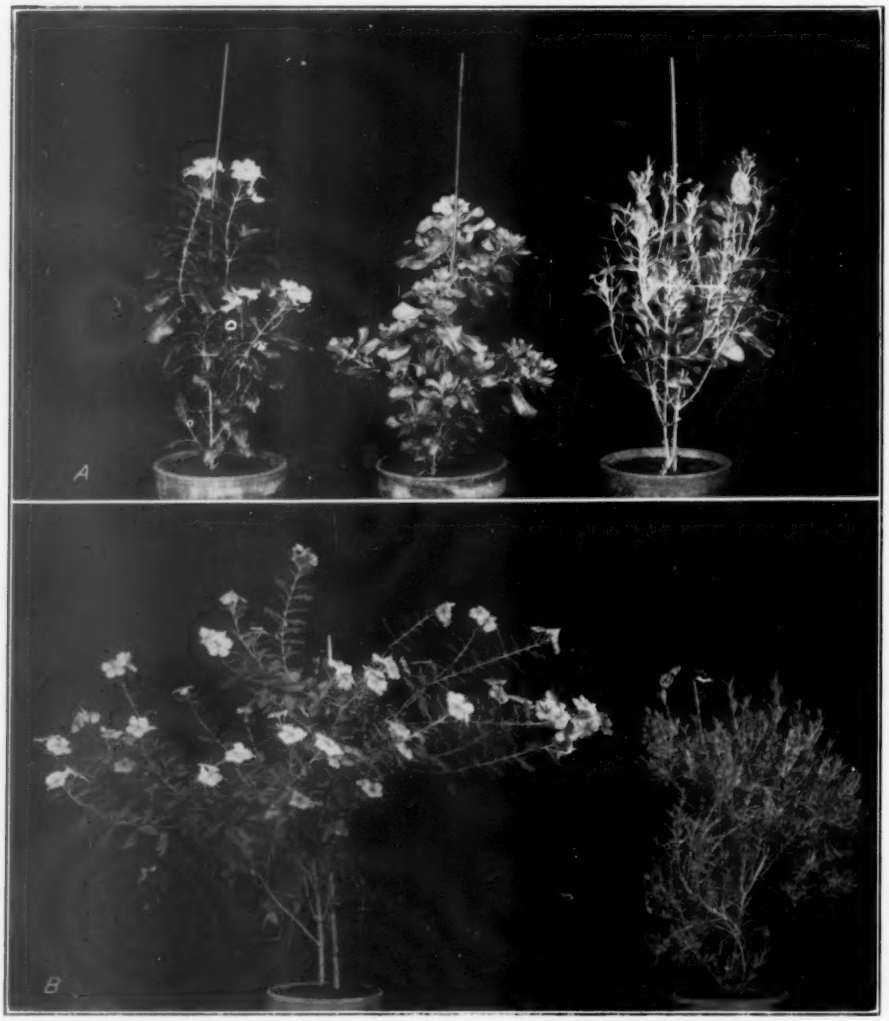

FIG. 2. A. Three periwinkle plants of the same age. Plant at the left is healthy, plant in the middle is affected by a mild strain, and plant at the right by a severe strain of false-blossom virus. B. Two periwinkle plants of the same age. Both were badly affected by false blossom when the plant at the left was given a 21-day treatment at 42° C. The picture was made about 5 months after treatment; it shows that the treated plant was cured and that the untreated plant remained badly diseased. (Photographs by J. A. Carlile.)

Juice was pressed out of leaves and stems of a false-blossom tomato plant and immediately rubbed over the upper surfaces of all leaves of 6 healthy tomato plants and 2 healthy plants of *Nicotiana glutinosa*. Juice from a healthy tomato plant was rubbed over the upper surfaces of the

leaves of 2 healthy tomato plants and 1 healthy plant of *Nicotiana glutinosa* that served as controls. All of the plants remained healthy in appearance during 3 months that they were kept under observation. It was concluded that the virus could not be transmitted to these species readily, if at all, by means of juice.

FALSE BLOSSOM NOT TRANSMITTED BY MACROSTELES DIVISUS (UHL.)

Although false blossom is a yellows type disease it seemed unlikely that it would be transmitted by the aster yellows vector, *Macrosteles divisus*. Nevertheless this possibility was tested in 3 experiments. In one of these, 65 virus-free nymphs were allowed to feed on a false-blossom carrot plant for 12 days. A similar virus-free colony of the same size was kept on a healthy carrot plant for the same period. The 2 colonies were held on 2 different aster plants for 5 days. Then each colony was passed successively to 11 different healthy carrot plants. They were confined on the plants for intervals varying from 2 to 12 days during a period totaling 40 days. Only 3 insects from the colony that had fed on the false-blossom carrot plant, and only 5 insects from the control colony were living when the test was ended. All plants were observed for 3 months following exposure to insects. They all remained healthy. It was concluded that *Macrosteles divisus* is unable to transmit cranberry false blossom in carrots.

FALSE BLOSSOM NOT TRANSMITTED THROUGH DODDER SEEDS

Seeds were collected from a large dodder plant that had grown and produced seeds on a false-blossom tomato. Many of the seeds were shrivelled and more or less deformed, but some were plump. Whether the poor quality of the seeds produced was due to infection of the dodder by false blossom virus or to insufficient nourishment furnished by the diseased host is not known. One hundred of the best-looking seeds were selected and planted in moist peat; only 6 of them germinated. When the seedlings were about one and one-half inches high each was transferred to a different healthy young tomato plant. All of the dodders grew and parasitized the plants on which they had been placed. After they had fed on the plants for 3 months they were removed and destroyed. The tomato plants were cut back and kept for 4 months after the dodders were removed. All remained healthy. Although the number of seedlings grown and tested was small, it was concluded that the virus cannot be transmitted readily, if at all, through dodder seeds.

An effort was made to determine whether it might be transmitted through tomato seeds. If Bonny Best tomato plants are large enough to be setting fruit at the time they become infected with false-blossom virus they will produce undersized fruits that ripen more or less normally. Such fruits usually bear no seeds but some contain fair numbers of small seeds and a few seeds that are almost but not quite as large as those borne by healthy fruits. Out of fruits that were harvested from 30 diseased tomato

plants 25 fairly normal looking seeds were obtained. They were planted in soil in two 8-inch pots. None of them germinated; hence it was not possible to determine whether or not they carried virus.

CURE OF FALSE BLOSSOM IN PERIWINKLES

A preliminary heat treatment experiment in which false-blossom periwinkles were held at 34° C. for periods up to 2 weeks gave no cures; hence, higher temperatures were tried. Eighteen diseased plants were divided into 9 sets of 2 plants each; 1 set was not treated and served as a control. The other 8 sets were treated at 36° C. for 7, 8, 9, 10, 11, 12, 13, and 14 days, respectively. A month after treatment it was observed that the tops of the plants exposed to heat for 7 and 8 days were somewhat greener than the tops of control plants, but that the new growth had symptoms of disease. The tops of the plants exposed for 9, 10, 11, and 12 days had produced new growth bearing normal flowers, and foliage that appeared healthy. However, sprouts that had developed on the main stems and large branches at some distance below their tips had false-blossom symptoms. The tops of the plants exposed for 13 and 14 days appeared healthy throughout, but sprouts arising at or slightly above the ground level showed symptoms in all cases. The tops of these plants apparently had been cured but virus had remained in the underground stem tissues, and, no doubt, in the roots.

In the next experiment 12 diseased plants were divided into 6 sets of 2 plants each. One set was not treated and served as a control. The other 5 sets were treated at 40° C. for 7, 8, 10, 12, and 14 days, respectively. The condition of the plants was observed and recorded 2 months after treatment. Those treated for 7 and 8 days had false-blossom symptoms in many of their branches but the tips of some branches bore normal flowers and healthy-appearing leaves. The tips of all branches on the plants exposed for 10 days were healthy in appearance, but the main stems and large branches bore diseased sprouts a few inches below the tips. The new growth in the tops of the plants treated for 12 days was healthy in appearance, but each plant bore diseased sprouts that arose near the ground level. The tops of these plants apparently had been cured but virus persisted in the underground parts. All new growth produced by the periwinkles treated for 14 days was healthy in appearance and remained so for the 8 months that the plants were under observation. It was concluded that they had been cured. The experiment showed that treatment of 40° C. for 12 days cured the tops but not the underground parts, while treatment at 40° C. for 14 days cured both tops and underground parts.

In another experiment diseased plants were treated at 42° C. for 14 days or longer. Ten diseased plants were divided into 5 sets of 2 plants each. One of the sets was not treated and served as a control; the other 4 sets were treated for 14, 16, 18, and 21 days, respectively. All of the plants were observed for 8 months. At no time did symptoms of disease

appear in new growth from the treated plants. The control plants were diseased throughout (Fig. 2, B). It was concluded that all of the treated plants in this experiment were cured.

CURE OF FALSE BLOSSOM IN CRANBERRIES

As has already been stated, preliminary heat treatment experiments with false-blossom cranberries were carried out soon after diseased plants were received. In one experiment 8 plants were exposed to 36° C. for periods varying by one day intervals from 7 to 15 days. None of the plants was seriously injured but none was cured. In another experiment 4 plants were treated at 38° C., and 4 at 40° C., for periods varying by 7-day intervals from 1 to 4 weeks. All of the plants treated for 2, 3, and 4 weeks were seriously injured and later died. The plants treated for one week lived and after some time produced new growth in which false-blossom symptoms eventually appeared.

When the results with periwinkles became available further heat treatments with cranberries were undertaken. The early cranberry experiments had indicated that heat treatments at fairly high temperatures for periods up to about 10 days caused less injury than treatments at somewhat lower temperatures for longer periods. The periwinkle experiments had shown that treatment at 40° C. had to be continued for about 2 weeks to inactivate all of the virus in underground parts. Therefore, treatments at 42° and 43° C. were tried.

While the work was in progress Stevens (26) published a brief note describing unsuccessful attempts to cure false blossom. He subjected dormant false-blossom plants to temperatures varying from 118° F. to 129° F. His periods of treatment varied from 5 minutes to 2 hours for the higher temperatures and up to 12 hours for the lower temperatures. He states that temperatures above 122° F. for periods longer than 30 minutes killed or hopelessly stunted the plants, whereas temperatures below 122° F. even after periods up to 3 hours or more failed to injure the virus.

In the first experiment diseased cranberries were held at 42° C. Three plants were treated for 5 days, 3 for 8 days, 3 for 9 days, and 3 for 10 days. Two of the plants treated for 9 days and 2 treated for 10 days died; all of the others lived and were held under observation for 2 years. The 3 plants treated for 5 days were not cured, as was shown by cranberry false-blossom symptoms that appeared in the new growth, and by transmission of virus from each to tomato plants. The other 5 plants showed no symptoms of false blossom (Fig. 3, A) in growth that developed after treatment, and dodder that was allowed to parasitize them did not transmit virus to tomato plants.

In another experiment 12 diseased cranberries were treated at 43° C. for one week. The plants were injured somewhat and 3 died about a month following treatment. The other 9 plants were held under observation for 2 years. Two of them showed symptoms of false blossom in new growth

about 10 months after treatment, and dodder allowed to parasitize them transmitted false-blossom virus to tomato. The other 7 plants did not show symptoms of false blossom, and dodder allowed to parasitize them did not transmit virus to tomato. Cuttings made from each of them produced healthy-appearing plants.

In another experiment 4 diseased plants were treated at 43° C. for 9 days, 4 for 10 days, and 4 for 11 days. All of the plants were rather seriously injured and most of them died. However, 2 treated for 9 days, 1 treated for 10 days, and 1 treated for 11 days survived. These plants

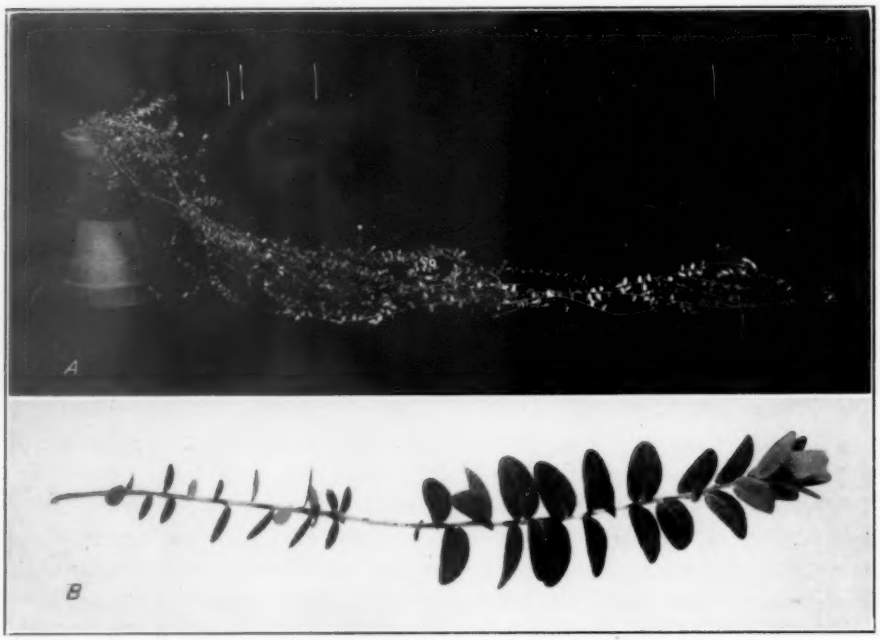

FIG. 3. A. Cranberry plant cured of false blossom by a 10-day treatment at 42° C. The picture was taken about 14 months after treatment. B. Cranberry branch showing recovery from false blossom following a 9-day treatment at 43° C. The small leaves on the older portion of the branch were produced before treatment, the large leaves on the tip portion after treatment. (Photographs by J. A. Carlile.)

showed no symptoms of false blossom in new growth in the 2 years that they were held under observation, and dodder allowed to parasitize them did not transmit virus to tomatoes. The portions of the vines produced by these plants before treatment bore small narrow leaves such as are produced by plants with false blossom, while the tip portions bore large broad leaves such as are produced by healthy plants (Fig. 3, B).

In the three experiments described a total of 16 false-blossom cranberry plants that were treated for varying periods at 42° and 43° C. seem to have been cured. It is known that certain viruses, as for example peach yellows, may be present in the lower parts of plants for a long time without moving into the tops (12). Even tobacco-mosaic virus is slow in

moving from roots into tops of tobacco plants (21). The possibility that false-blossom virus may have remained in the underground portions in some or all of the cranberry plants that apparently were cured must be admitted. The aboveground portions of the plants undoubtedly were cured.

DISCUSSION

Although cranberry false blossom has been known to science for about thirty-five years and is of considerable economic importance (25) the nature of the disease was not fully established until 1929, when Dobroscky (5) found that it was caused by a virus transmitted by the blunt-nosed leafhopper *Euscelis striatulus* (Fallen). Previous to that time false blossom had been thought by some to result from a physiological disturbance, possibly a disturbance in nitrogen nutrition (24). Dobroscky (6), using the leafhopper vector, attempted to transmit false blossom to the blueberry, *Vaccinium corymbosum* L., but did not succeed. Up to the time dodder transmission was reported in 1942 the disease had not been seen on any species except the American cranberry, *Vaccinium macrocarpon* Ait., and the European cranberry, *Vaccinium oxycoccus* L. (15). It has now been transmitted by dodder to species belonging in ten different families of plants. Thus another plant virus that, for a long time, was thought to be highly specific has been found capable of infecting many species that are not closely related to the natural host. Whether the disease in nature occurs on any of these plants is not known with certainty, but it seems probable that a tomato disease prevalent in Australia and generally known under the name of big bud is identical with or closely related to false blossom. Samuel, Bald, and Eardley (23) state that "The disease that presents the closest parallel to tomato big bud as regards the host plant is undoubtedly cranberry false blossom, transmitted by the jassid *Euscelis striatulus.*" At the time this statement was made false blossom had not been taken to tomato; hence it was not known that it would produce typical big-bud symptoms. The Australian disease is spread by the leafhopper *Thamnotettix argentata* (Evans) (8). Similar big-bud diseases of tomato also occur in the western part of the United States (4) and in Russia (22), where they are known under the names big bud and stolbur, respectively. The vector or vectors of these diseases are unknown. Another similar disease affects eggplant in southern India, where it has been reported under the name of little leaf (9). It is transmitted by the leafhoppers *Empoasca devastans* Dis. and *Eutettix phycitis* Dis. Hill (7) states that "It seems likely that the diseases known as 'big bud' in Australia, 'stolbur' and 'montar' in the U.S.S.R. and 'little leaf' in South India, are all due to the same virus or to very closely related viruses." The symptoms produced by big bud of tomato and by false blossom in tomato in the United States suggest these diseases also belong in the stolbur group. They probably are produced by closely related strains of one virus. Several plants on which big bud in Australia have been found

occurring naturally or to which it has been transmitted have been shown to be susceptible to false-blossom virus. These include tomato, tobacco, parsley, parsnip, cultivated carrot, celery, *Phlox Drummondii* Hook., and *Calendula officinalis* L. Both viruses are known to affect species in the genera *Petunia, Centaurea, Gaillardia,* and *Dianthus.* Thus two of the viruses in the group show similarities in host ranges as well as in symptoms produced. False blossom causes woodiness in fruits and stems similar to that described by Michailowa (20) for stolbur in tomato. These two viruses produce histological changes that are closely parallel. It is also interesting to note that of the four leafhoppers known to spread diseases of this type, three, *Euscelis striatulus, Eutettix phycitis,* and *Thamnotettix argentata,* are in closely related genera, while the fourth, *Empoasca devastans,* is in a closely related division of the subfamily Jassinae.

False blossom is the first disease in the group to be cured by heat. If the other diseases are as closely related as is believed they also should respond to heat treatments. Although false blossom has been cured only in the periwinkle and the cranberry it seems likely that it might be cured in many other species. Whether the heat cure described in this paper will be of any practical value to cranberry growers is not known. If some method could be found of warming up the water in flooded cranberry bogs it might be possible to cure the tops of diseased plants at least.

It is not known why some cranberry plants were cured by treatment at 43° C. for 7 days, while others were not. However, this was the mildest treatment that proved effective and it is possible that the plants which retained virus had somewhat thicker stems or were rooted more deeply than those that were cured. Judging by results of the experiments reported in this paper treatments at 42° or 43° C. for about 8 days are to be recommended for cure of cranberry plants. False-blossom virus proved to be somewhat less sensitive to heat than the viruses of aster yellows (14), peach yellows (13), or potato witches'-broom (18).

SUMMARY

1. Cranberry false-blossom virus was transmitted by dodder, *Cuscuta campestris,* from cranberry to 28 different species of plants belonging in 10 different families. It was transmitted by dodder from *Vinca rosea* and tomato to cranberry plants. It was retained by dodder growing on healthy plants over a period of two years, and apparently multiplies in this vector.

2. False-blossom virus also was transmitted to *Vinca rosea,* Turkish tobacco, tomato, potato, *Nicotiana glutinosa,* and *N. rustica* by grafting.

3. It was not transmitted mechanically by means of juice, by the aster leafhopper, *Macrosteles divisus,* or through dodder seeds produced by the parasite while growing on a diseased tomato plant.

4. In all the species to which it was taken false blossom produced yellows type symptoms. In many it caused gigantism in flowering and fruiting organs; it caused sterility in the tomato.

5. False blossom in periwinkle and cranberry was cured by heat treat-

ments. Treatments at 42° or 43° C. for about 8 days are recommended for cure of cranberry plants.

6. It is suggested that false blossom may be closely related to big bud of tomato in Australia and the United States, to stolbur of tomato in the U.S.S.R., and to little leaf of eggplant in South India.

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A LEAF SPOT OF COWPEA AND SOYBEAN CAUSED BY AN UNDESCRIBED SPECIES OF HELMINTHOSPORIUM

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(Accepted for publication May 25, 1945)

On August 14, 1944, several leaves of *Vigna sinensis* (L.) Endl. with leaf spots of a type not heretofore observed on this plant were collected from a severely infected field near La Place, Louisiana, and examined for pathogens. The only organism constantly associated with these spots was a species of *Helminthosporium* which does not appear to have been previously described. At La Place, the fungus was causing a great deal of damage to several varieties of cowpea over an area of 50 acres. It was not found by us in any other locality in the State, nor on any plant other than the cowpea in that area.

Later, we observed a note in the Plant Disease Reporter (Vol. **28**: 970. October 1, 1944) to the effect that an unidentified species of *Helminthosporium* had been found on cowpea leaves by the Bureau of Entomology and Plant Quarantine. At our request, Dr. D. P. Limber generously sent us specimens of this material. Three collections, all made by A. W. Blizzard, were examined. Two of the collections—one from Burton, South Carolina, the other from Elizabeth City, North Carolina—were found to be identical with our fungus. A third collection from Seabrook, South Carolina, is still another species which we do not find listed for cowpea or any other leguminous host.

On looking over some diseased soybean specimens collected in Florida during 1943, a few leaves of Mamredo and Edsoy varieties collected in August had several leaf spots caused by a fungus which appeared to be identical with the one on cowpea. A culture of the fungus, made in August, 1943, was found to be still viable.

The only species of *Helminthosporium* reported on *Vigna sinensis* up to the present time is *H. molle* B. & C., which is entirely different from the species described in this paper. After a search through the literature, in which particular attention was given to all descriptions of species on leguminous hosts, we were unable to find any description of the fungus studied by us. Moreover, no previous report of a *Helminthosporium* on soybean was found.

The finding of this fungus primarily in endemic areas near ports of entry would seem to indicate that it has been recently introduced into the United States from some other country.

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DESCRIPTION OF THE LEAF SPOT

This disease, to which we are giving the common name of target spot, is chiefly one of the foliage of cowpeas, where it first appears as numerous reddish-purple dots which gradually enlarge into conspicuous brown circular areas, often with wavy margins (Fig. 1, A). Mature spots almost always have an outstanding zonation, consisting of a varying number of reddish-brown rings against a lighter brown background (Fig. 1, B). Each spot generally has a small reddish-brown dot in the center. Several spots may coalesce to produce more extensive brownish areas on the leaf, but each one maintains its own identity by virtue of its conspicuous zonation and the

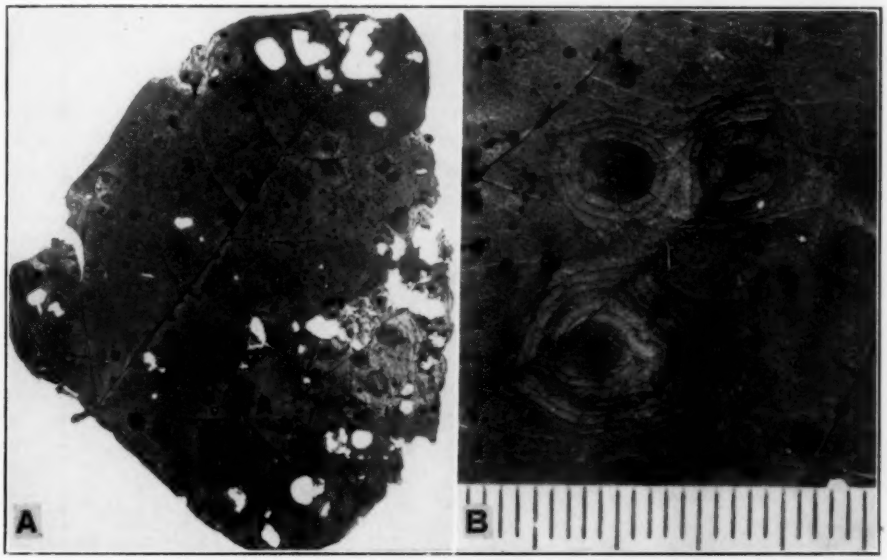

FIG. 1. Cowpea leaves showing natural infection with *Helminthosporium vignae* sp. nov. A. Leaflet with characteristic spots and shot-hole effect. B. Enlarged spots, showing concentric zonation.

central dot. The spots show on both surfaces of the leaf, but are more conspicuous on the upper surface. Eventually the dead brownish tissue may rupture and fall out completely, leaving holes in the leaf and imparting to the leaf an appearance of having been eaten out by insects (Fig. 1, A). Yellowing of the entire leaf and defoliation occur in severest cases. Most of our leaf specimens from the field have from 2 or 3 to an indefinite number (50 or more) of zoned spots and a larger number of small, purplish, undeveloped spots. The majority of zoned spots are 3 to 10 mm. in diameter, but some range up to 2 centimeters.

On diseased soybean from the field, a few less conspicuous brown spots caused by the fungus were found scattered over a few of the leaves. These spots did not have the zonation so characteristic of those on cowpeas. Damage to the soybean by this fungus does not seem to be serious.

STEM INFECTIONS

Later in the season it was observed that the fungus was capable of producing numerous reddish-purple spots and streaks of various sizes on mature stems and petioles of cowpeas already made unhealthy by severe foliage infection (Fig. 2). The *Helminthosporium* was found sporulating on some

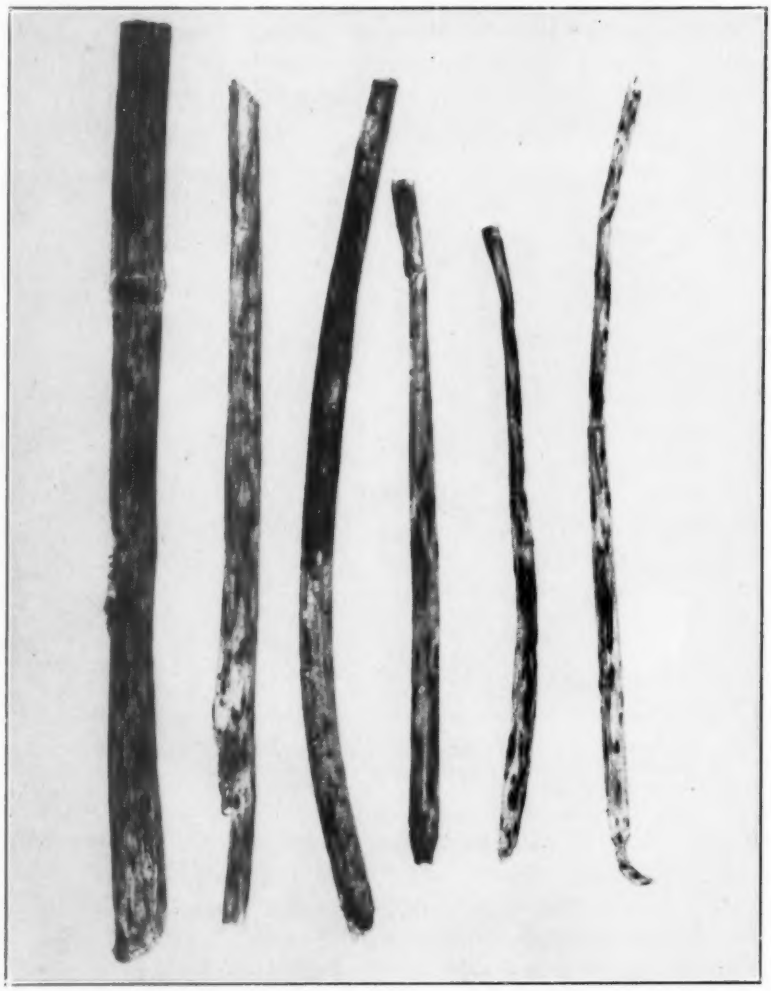

FIG. 2. Stems and petioles of cowpeas naturally infected with *Helminthosporium vignae* sp. nov. Natural size.

of these spots. On the other hand, we obtained almost no stem infection from inoculations of young cowpea plants in the greenhouse, and no visible damage resulted from the few small and rather superficial purplish spots which did appear on the stems. Thus it appears that only old stems of unhealthy cowpeas are likely to be attacked with any severity by the fungus.

Other fungi were generally associated with the purple stem spots of field specimens and were undoubtedly partly responsible for the severity of the stem infections.

No spotting of soybean stems by this fungus has been observed.

DESCRIPTION OF THE FUNGUS

When diseased cowpea leaves are brought in from the field and examined under low power, numerous elongate, dark brown conidiophores may generally be seen on both surfaces of the leaf spot. Conidiophores tend to be more abundant on the reddish-brown rings than between them. They occur either singly or in groups of 2 to 6.

Under high-power magnification the conidiophores are 1–20-septate and variable in length, measuring 6–11 × 44–380 μ. The majority are 3–5-septate and 8 × 125–200 μ. They arise from swollen basal cells and produce conidia singly or in chains at their tips (Fig. 3, B and C). Rarely a condiophore may grow beyond the point where the first conidium was formed to produce a second one (Fig. 3, D).

When the cowpea and soybean leaves are first brought into the laboratory for examination, the conidia present on the leaf spots are typically brownish, elongate, broadest at the base and very perceptibly tapered towards the apex (Fig. 3, A). They measure 8–19 × 40–270 μ and are 3–20-septate, the average being about 17 × 120 μ and 10-septate. They may be straight or curved, the latter characteristic applying to most of the longer ones. Some are long-cylindrical and of almost the same diameter throughout (Fig. 3, D). Their walls are thickened in the manner characteristic for the genus.

When diseased leaves are placed in a moist chamber, the conidiophores tend to be slightly longer, but do not vary significantly from the description given. However, few of the conidia formed in the moist chamber have the broad base and tapering apex which are typical of those produced in the field. They are generally straight or somewhat curved, long and slender, and usually taper very gradually, if at all, towards the apex (Fig. 3, B and E). They measure 7–11 × 40–306 μ and are 1–20-septate, averaging 10 × 150–250 μ and 10–15-septate. They appear in chains of 2–5. This tendency towards catenulation has been frequently observed in the field early in the morning while the dew still covers the plants. It will be described more fully under the discussion of cultural characteristics.

The conidium generally becomes multiseptate before it is shed. On germination it produces a germ tube at each end (Fig. 3, E). At the basal end there is a hilum, containing a centrally located pore through which the germ tube passes (Fig. 3, A, E, and F). The other germ tube passes directly through the apex of the conidium where the wall is very thin (Fig. 3, E). A few conidia have been seen to produce an additional germ tube from an intermediate segment, but this is rare.

When diseased stems are placed in a moist chamber numerous conidiophores bearing conidia appear on them. Measurements fall within those

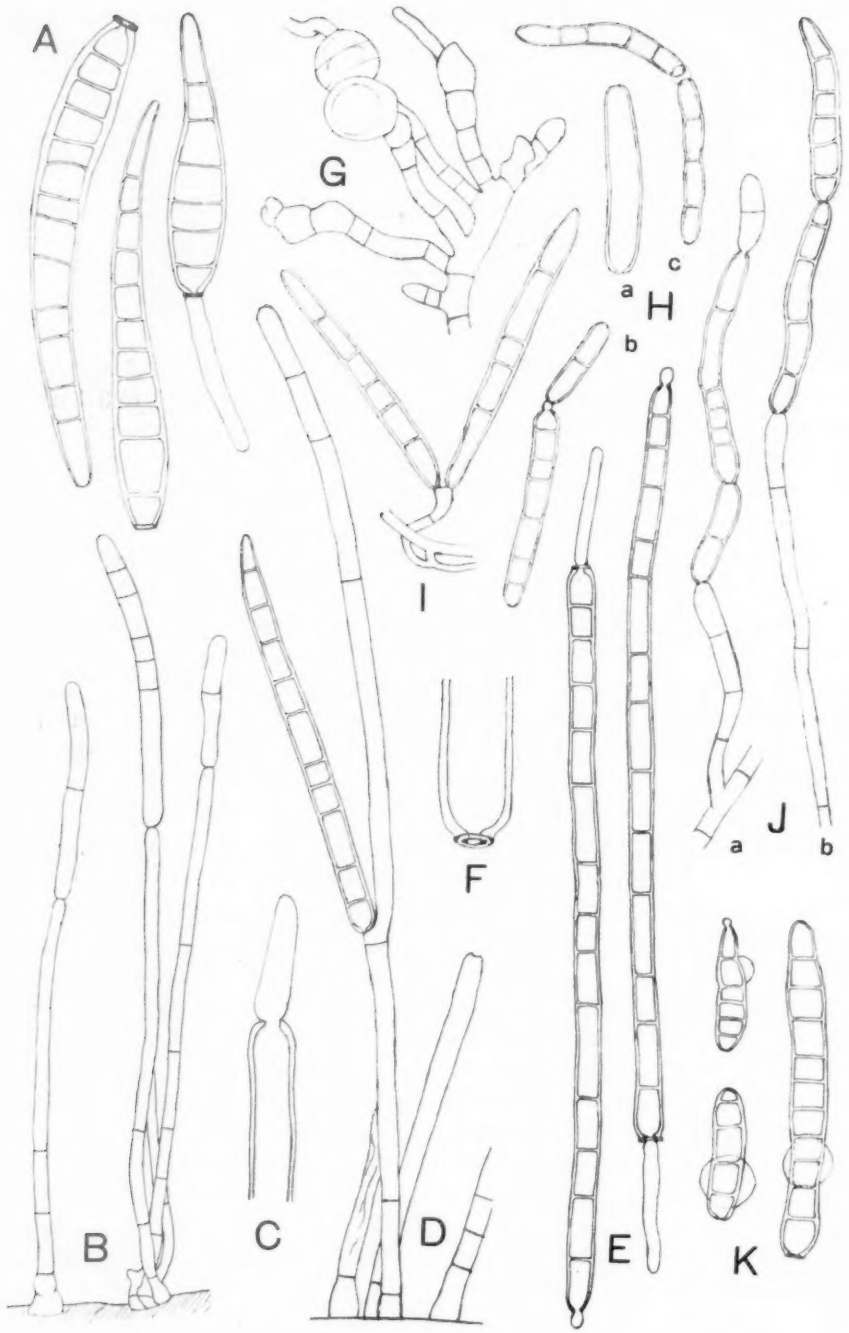

FIG. 3. *Helminthosporium vignae* sp. nov. A–E. Conidiophores and conidia from diseased cowpea leaves. F. Hilum end of conidium. G. Chlamydospores from agar culture. H–K. Conidia and conidiophores from agar culture. All figures ×340, except B, ×225, and C and F, ×710.

already given for the fungus on the leaf spots: most of the condia are 8–11 × 86–196 μ and are 4–7-septate, while the conidiophores reach their maximum length here, measuring 6–8 × 34–750 μ.

No definite morphological differences between the fungus on cowpea leaves and stems and the one on soybean leaves have been observed. Certain physiological differences have been determined, however, and these will be described later in connection with inoculation results.

CULTURAL CHARACTERISTICS

Single-spore cultures of the fungus were made and its growth rate and degree of sporulation tested on potato-dextrose, corn-meal, prune, malt, and Czapek's-solution agars. It grew and fruited on all of these, but the best production of conidia occurred on potato-dextrose and on Czapek's-solution agar, the poorest on malt agar. In most cases the rate of growth is moderate and conidia begin to appear about 5 to 6 days after the culture is started. The hyphae at first form a white flocculent mass, which later becomes dark gray and forms an olivaceous black mat at the surface of the agar. This dark discoloration eventually extends throughout the agar medium. Hyphae and conidia at first appear nearly hyaline under the microscope, but soon become dusky brown.

In culture the conidia are typically borne in chains of 2–5, or possibly more (Fig. 3, J). They appear to be produced acropetally only; that is, the first conidium produces at its apex a secondary conidium, which in turn may give rise to a third in the same way and so on. Frequently as many as 3 are found in a row before any of them have matured. Often they begin to germinate before they separate from the chain, in which case the developing germ tubes impart to the conidia the appearance of being connected by little intercalary plugs (Fig. 3, H, b). This same tendency towards catenulation was also observed by Drechsler[4] in *Helminthosporium catenarium* Drechs. and by Berg[5] in *H. papulosum* Berg.

Frequently hyaline vesicles, probably gelatinous in nature but with function unknown, are found in various positions on the conidia. A vesicle is sometimes at the point of contact of two conidia in a chain (Fig. 3, H, c); at other times it is around some of the intermediate segments (Fig. 3, K). Similar structures were also reported by Berg for *Helminthosporium papulosum*. Our fungus has several characteristics which seem to ally it with the species studied by Berg, the catenulate nature of the conidia in a moist environment and the occurrence of vesicles on them in culture being two of the most important similarities. The vesicles were not observed on conidia produced on leaf and stem spots in nature.

Rarely, a conidiophore produced in culture bears two conidia at its tip (Fig. 3, I). While most of the conidia are 4–8-septate, they may range from

[4] Drechsler, C. Some graminicolous species of *Helminthosporium*: I. Jour. Agr. Res. [U. S.] 24: 670. 1923.

[5] Berg, A. Black pox and other apple-bark diseases commonly known as measles. West Virginia Agr. Exp. Stat. Bull. 260. 1934.

unicellular to 15-septate and measure 7–12 × 26–204 μ in culture on potato-dextrose or Czapek's-solution agar (Fig. 3, H–K). Some are broader at the base and taper conspicuously towards the tip, but the majority are cylindrical and about the same width throughout. They vary from straight to conspicuously curved. Conidiophores in culture are mostly 1–4-septate and extremely variable in size, measuring 4.5–8 × 26–440 μ.

Soon after the culture becomes well established, numerous chlamydospores generally appear. They are most abundant in older cultures and cultures made by repeated transfers. They are hyaline, terminal or intercalary, and measure 14–20 × 16–30 μ.

With respect to cultural characteristics, no essential differences were observed between the fungus isolated from cowpea leaves and the one from soybean leaves.

INOCULATION EXPERIMENTS

Cultures of the fungus isolated from cowpea leaves collected in Louisiana and from leaf spots of Mamredo soybean collected in Florida, were used in making inoculations. These inoculations consisted of spraying the plants with an aqueous suspension of spores and hyphae of the fungus. The pathogenicity of the fungus from cowpea was tested on four varieties of cowpea (Blackeye, Early Buff, Early Silver Crowder, and Iron) and on six varieties of soybean (Nanda, Avoyelles, Otootan, Biloxi, Seminole, and Mamredo). All varieties of cowpea showed signs of infection within 3 days following inoculation. In 6 days the results were outstanding. Many conspicuous purple spots appeared on the leaves, followed by a yellowing of the more severely infected leaves. Considerable defoliation was the final result. The variety of cowpea most severely attacked was Iron; the least injured was Blackeye. Damage to Early Buff and Early Silver Crowder was severe.

Leaf spots appearing on inoculated cowpeas eventually turned brown, but each retained a purple border and a purple dot in the center. These spots did not have the concentric zonation found on diseased leaves collected in the field (Fig. 4, A). The fungus was readily recovered from the spots induced by inoculation.

All 6 varieties of soybean showed slight infection in the form of small, scattered, purplish spots which appeared about 3 days after inoculation and had little or no tendency to enlarge. These spots often had a light yellow discoloration around the border. They became brown, but remained small and without concentric zonation. Since the infection on soybean was light, no defoliation resulted. The fungus was recovered from leaf spots on these inoculated soybeans.

In spite of its morphological similarity to the fungus isolated from cowpea, the fungus isolated from Mamredo soybean did not give the same results on inoculation. The Iron cowpea, which was so heavily spotted by the former, was only slightly infected when inoculated with the latter. Few to many small purple specks appeared on the leaves, but these tiny spots

showed little tendency to enlarge and caused little or no permanent damage to the plants. When several Biloxi and Mamredo soybean plants were inoculated with the fungus, numerous, small, reddish-purple spots developed (Fig. 4, B). These spots showed little tendency to enlarge and eventually turned brown. They were very similar to those produced by the fungus from cowpea leaves. No defoliation occurred and damage to the plants was

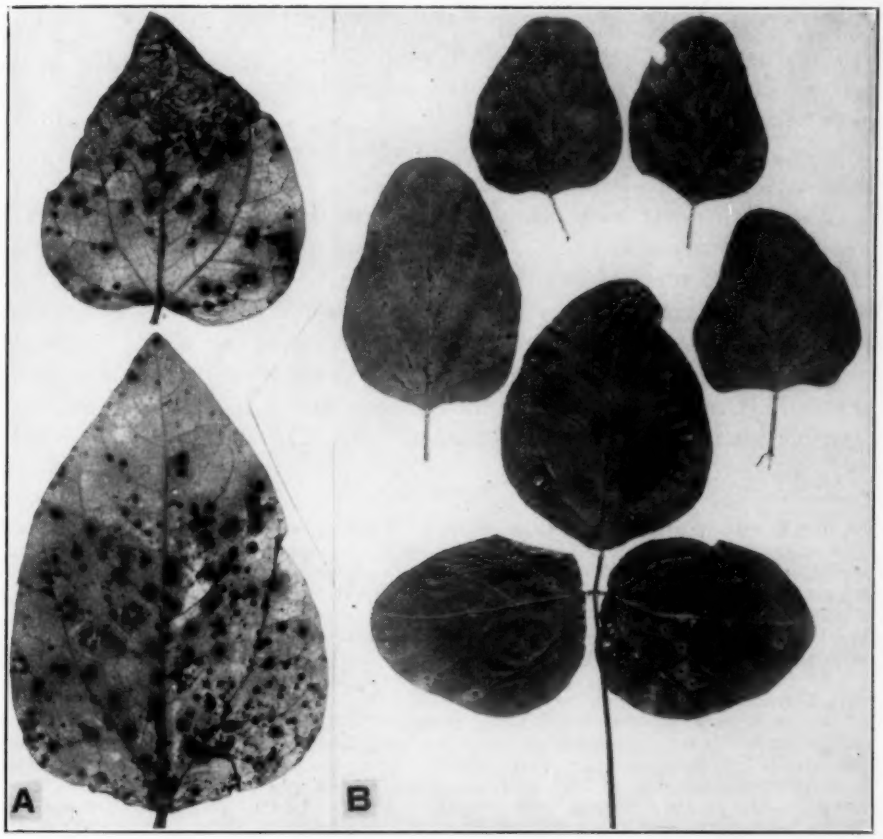

FIG. 4. Results of artificial inoculation with *Helminthosporium vignae* sp. nov., showing spots on leaves of cowpea (A) and soybean (B).

almost negligible. The fungus was recovered from leaf spots on inoculated soybeans and from a few spots on inoculated cowpeas.

It is apparent that two parasitic races of the *Helminthosporium* have been involved in these investigations. Race 1, isolated from cowpea leaves, is capable of causing severe infection of cowpeas and a light spotting of soybeans. Race 2, isolated from leaves of Mamredo soybean and morphologically similar on the host tissue as well as in culture to Race 1, produces a light spotting of soybean leaves and few to many very small spots on the cowpea, with little or no damage resulting to either host.

It was discovered that, after 3 or 4 transfers of Race 1 had been made, this race of the fungus lost its pathogenicity and failed to produce any infection on either cowpea or soybean. Very often conidia ceased to appear after repeated transfers of the fungus had been made. However, loss of pathogenicity does not appear necessarily to be related to a lag in conidial production, since some old cultures which were still producing conidia had lost their virulence. The most effective inoculum was made from cultures grown directly from single conidia taken from the diseased leaf, without any intervening transfers. Race 2 seemed to retain its pathogenicity better. All cultures of it used in inoculations came originally from a culture which had been isolated in August, 1943. It produced conidia abundantly and proved to be as pathogenic to soybeans as Race 1 after we rejuvenated it by growing it on oats and then placing the oats on agar.

The only sign of stem infection in any of the inoculation experiments was the appearance of a few rather superficial purplish spots on the stems of some of the cowpeas inoculated with Race 1. These spots became less conspicuous as growth of the plants continued, and no damage was caused by them. The fungus was not found sporulating on any of the leaf spots resulting from inoculations until diseased leaves were placed in a moist chamber. Conidia produced on these leaves tended to be longer and less tapering than those already described (Fig. 3, E).

DIAGNOSIS[6]

HELMINTHOSPORIUM **vignae** Olive, sp. nov. Maculae in foliis Vignae primum punctiformes, parvae et purpureae, dein majores, brunneae, conspicuae, concentrice zonate, demum secedentes, in foliis Sojae minores, brunneolae, ezonatae; conidiophora amphigena, singula vel 2–6-caespitosa, fusca, 6–11 × 44–380 (–490) μ, 1–20-septate, typica 8 × 125–200 μ et 3–5-septata; conidia fusca, acrogena singulata vel catenulata oriunda, recta vel curvata, basi lata, apice insigniter attenuata, interdum anguste cylindrica, 8–19 × 40–270 μ, 3–20-septata, typice 15–18 × 100–180 μ et circa 10-septata.

In foliis caulibusque *Vignae sinensis* et foliis *Sojae max* in Carolina boreali et australi, Florida, et Louisiana.

Spots on leaves of cowpea beginning as small purple dots and enlarging to conspicuous concentrically zoned brown spots which are often deciduous; stems with purplish spots and streaks late in the season; spots on leaves of soybean smaller, brownish, not zoned. Conidiophores appearing on both surfaces of the leaf, singly or in groups of 2–6, dark brown, 6–11 × 44–380 (–490) μ, 1–20-septate, typically 8 × 125–200 μ and 3–5-septate; conidia brown, produced singly at the tips of the conidiophores, becoming catenulate under very moist conditions, straight or curved, with broad base and conspicuously tapering apex, sometimes varying to narrow cylindrical, 8–19 × 40–270 μ, 3–20-septate, typically 15–18 × 100–180 μ and about 10-septate, germinating by means of two polar germ tubes, the basal one passing directly through a pore in the center of the hilum.

In culture on Czapek's-solution agar or potato-dextrose agar, conidia appearing within 5–6 days, and flocculent mycelium, white at first, becoming deep gray. Conidiophores mostly 1–4-septate and very variable in size, 4.5–8 × 26–440 μ; conidia cylindrical or tapering towards apex, often curved, typically in chains of 2–5, measuring 7–12 × 26–204 μ, 0–15-septate; chlamydospores becoming numerous in older cultures, hyaline, mostly around 14–20 × 16–30 μ.

On leaves, or on stems late in the season, of *Vigna sinensis* (L.) Endl., and on leaves of *Soja max* (L.) Piper; North Carolina, South Carolina, Florida, and Louisiana.

[6] The authors wish to thank Miss Edith Cash for preparing the Latin diagnosis of the fungus described in this paper.

Specimens of diseased cowpeas and soybeans have been deposited in the Mycological Herbaria at the Beltsville Plant Industry Station and at Louisiana State University.

SUMMARY

A species of *Helminthosporium*, apparently heretofore undescribed, has been found to cause a severe leaf spotting of cowpeas, with stem infections taking place late in the season. This fungus also causes a light spotting of soybean leaves. Two parasitic races have been isolated. Race 1, isolated from cowpea leaves, causes a severe leaf spotting of cowpeas and a light spotting of soybeans; while Race 2, isolated from soybean leaves, produces a light spotting of soybean leaves and few to many small specks of little consequence on cowpea leaves.

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PARASITISM OF RHIZOCTONIA SOLANI FROM ALFALFA[1]

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(Accepted for publication May 25, 1945)

In a previous publication (12) the writer described a root canker of alfalfa, which occurs in southwestern Arizona and southern California, and attributed its cause to *Rhizoctonia solani* Kühn. This fungus species is known to have a wide host range (2, 3, 9), and strains within the species have been recognized by several investigators (5, 6, 7, 10, 11). This publication reports results of pathogenicity tests on alfalfa with random isolates of *R. solani*, and tests of the pathogenicity on other crop plants, of the strain which causes alfalfa root canker.

METHODS AND MATERIALS

Plants were grown in galvanized iron cans, 7 inches in diameter and 11 inches deep. A sandy loam soil from Nevada was used with its natural microflora, except for the addition of a small amount of inoculum. Inoculum was prepared by growing *Rhizoctonia solani* for about two weeks on previously soaked and sterilized barley grain. When the plants had a good top growth, five holes, about ¼ inch in diameter and 9 inches deep, were made in each can of soil and the holes filled with inoculum. The temperature of the soil was then kept at 29–30° C. Usually the plants were exposed to the organism for about 4 weeks before they were removed from the soil and their roots examined for lesions. In some cases, however, disease development was rapid enough to allow for root examination after a shorter period.

All results herein reported on alfalfa were obtained with the southern grown variety California Common.

PATHOGENICITY OF ISOLATES FROM ALFALFA TO ALFALFA

Isolates of *Rhizoctonia solani* were obtained from alfalfa grown in California and Nevada. Some of the isolates were from root lesions, some from fine transient roots (4), and some from young damped-off seedlings. A list of the isolates used and results obtained when California Common alfalfa was inoculated with them are in table 1.

Only certain isolates produce root lesions on alfalfa (Table 1). Isolates obtained from alfalfa grown in Nevada caused no root lesions, whereas isolates from root lesions on alfalfa grown in California, where the root canker is prevalent, caused root lesions. Since one isolate from a transient root of alfalfa, grown at Bard, California, did not produce root lesions, it is evident that isolates which cause root lesions and isolates which do not cause root lesions may be obtained from areas where the disease occurs.

[1] Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Nevada Agricultural Experiment Station.

[2] Associate Pathologist, Division of Forage Crops and Diseases, U. S. Department of Agriculture.

PATHOGENICITY OF ISOLATES FROM OTHER PLANT SPECIES TO ALFALFA

To determine the pathogenicity of *Rhizoctonia solani* from other crop plants to alfalfa, isolates known to be pathogenic on certain plants were used, as well as some that had not been tested for pathogenicity. Isolates were obtained from sclerotia on potato tubers, and from underground lesions on potato stems. Others were obtained through the courtesy of W. J. Cherewick,[3] John E. Kotila,[4] and D. C. Neal.[5] Two isolates were furnished by Dr. Cherewick; one P–66, reported to be pathogenic on potatoes, and the

TABLE 1.—*List of isolates of Rhizoctonia solani and results obtained from greenhouse inoculation to alfalfa. All isolates obtained from alfalfa*

Isolate No.	Place where plant was grown	Part of alfalfa plant from which isolated	Inoculation results[a]
92	Minden, Nevada	Lesion below ground on stem of young seedling	0/28
122	do	.....	0/27
115	do	Root lesion	0/21
96	Reno, Nevada	Damped-off seedling in greenhouse	0/28
119	do	do	0/25
120	do	do	0/23
121	do	do	0/22
98	Bard, Calif.	Transient root	0/27
102	do	Root lesion	28/28
103	do	do	4/26
105	do	do	21/21
127	El Centro, Calif.	do	22/30
128	do	do	20/23
129	do	do	21/30
130	do	do	24/25
131	do	do	30/40
132	do	do	23/26

[a] Numerator = number of alfalfa plants with root lesions.
Denominator = number of alfalfa plants inoculated.

other, S.C.R.I., reported as causing a crown rot and seedling damping-off of sweetclover and alfalfa (1). Three isolates (R–167, R–216, and R–380) were furnished by Dr. Kotila; R–167 and R–216 are reported as causing damping-off and root rot of sugar beets, and R–380 as causing damping-off and foliage blight of sugar beets. One isolate, pathogenic on cotton leaves (8), was furnished by Dr. Neal. The results from inoculation tests on alfalfa plants with these isolates of *Rhizoctonia solani* are in table 2.

In only one instance (R–216) was *Rhizoctonia solani* from other crop plants capable of causing root lesions on alfalfa (Table 2). In this case 4 of 40 roots inoculated with R–216 had root lesions; one root had 3 lesions, and three roots had one lesion each. The test was repeated with 50 alfalfa

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[4] Pathologist, Division of Sugar Plant Investigation, U. S. Department of Agriculture, Beltsville, Maryland.

[5] Senior Pathologist, Cotton Disease Investigation, U. S. Department of Agriculture, University of Louisiana, Baton Rouge, Louisiana.

TABLE 2.—*Results obtained from greenhouse inoculation tests to alfalfa with isolates of Rhizoctonia solani from other plant species. Inoculated June 1, observed July 22, 1944*

Isolate no.	Host plant	Part of plant from which isolated	By whom isolated	Inoculation results[a]
R–116	Potato	Sclerotium on potato tuber	O. F. Smith	0/25
R–117	do	do	do	0/21
R–126	do	Underground lesion on potato stem	do	0/38
P–66	do	.....	W. J. Cherewick	0/50
S.C.R.I.	Sweetclover	Root	do	0/58
R–167	Sugar beet	.....	John E. Kotila	0/27
R–216	do	.....	do	4/40
R–380	do	.....	do	0/32
R–134	Cotton	Leaf	D. C. Neal	0/51

[a] Numerator = number of alfalfa plants with root lesions.
Denominator = number of alfalfa plants exposed to organism.

plants, using isolate R–216 of *R. solani*, and 8 of 50 plants inoculated had one or more lesions. Alfalfa plants inoculated at the same time with isolate 102, obtained from a root lesion on alfalfa at Bard, California, averaged 29.4 lesions per main root for 25 plants. Thus R–216 can be considered as being only very weakly pathogenic on alfalfa roots under the conditions of these experiments.

PATHOGENICITY OF RHIZOCTONIA SOLANI, ISOLATE 102, ON PLANTS OTHER THAN ALFALFA

Isolate 102 of *Rhizoctonia solani* is very pathogenic on alfalfa roots (Table 1). It seemed advisable to determine if this particular isolate is pathogenic on other crop plants, some of which are grown in southern California and southwestern Arizona where Rhizoctonia root canker is prevalent

TABLE 3.—*Results obtained in 1944 by inoculating several plant species with isolate 102 of Rhizoctonia solani from alfalfa*

Plant	Date		Inoculation results[a]
	Inoc.	Examined	
Canada field pea, *Pisum sp.*	Jan. 1	Jan. 14	20/41
Bard vetch, *Vicia calcarata* Desf.	do	do	35/35
Berseem clover, *Trifolium alexandrinum* L.	Mar. 17	Apr. 13	7/67
Guar, *Cyamopsis tetragonolobus* Taub. (*C. psoraloides* DC.)	do	do	6/25
Hubam sweetclover, *Melilotus alba* Desr.	do	do	19/40
Sour clover, *M. indica* All.	do	do	18/65
Madrid Evergreen sweetclover, *M. alba* Desr.	June 1	July 22	32/34
Cumberland red clover, *Trifolium pratense* L.	do	do	10/10
Alfalfa, *Medicago sativa* L.	do	do	33/33

[a] Numerator = number of plants with root lesions.
Denominator = number of plants exposed to organism.
Control plants of all species were treated exactly like inoculated plants except that no organism was growing on the barley grain added to the soil. All control plants remained healthy.

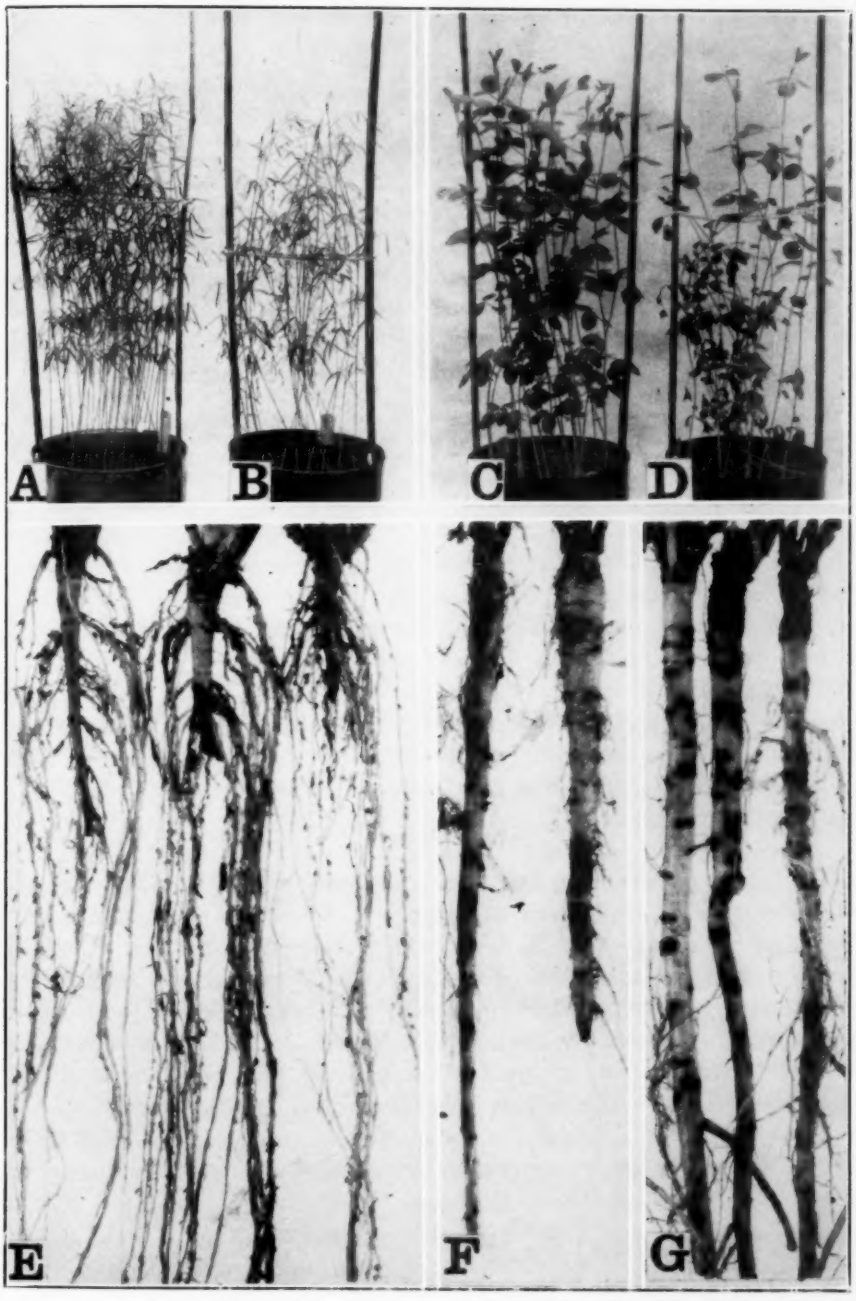

FIG. 1. Effect of *Rhizoctonia solani* on several plant species at a soil temperature of 29°–30° C. A, Bard vetch, not inoculated; B, Bard vetch, inoculated; C, Canada field pea, not inoculated; D, Canada field pea, inoculated; E, Cumberland red clover, inoculated; F, Madrid sweetclover, inoculated; G, Alfalfa, inoculated. A through D photographed two weeks after inoculation, E through G photographed approximately seven weeks after inoculation.

on alfalfa. Results from inoculation tests with isolate 102 on several plant species are in table 3.

Isolate 102 of *Rhizoctonia solani* is pathogenic on several plant species, but it is more pathogenic on some than on others. However, the effects of the organism on the different species are not alike. On alfalfa (Fig. 1, G), Madrid sweetclover (Fig. 1, F), and guar, the organism causes characteristic, circular, dark lesions on the main tap root and large lateral roots, but no marked symptoms on top growth until the disease has reached an advanced stage of development, at which time badly infected plants are somewhat stunted and some are killed. On Canada field peas, the organism invades the stem at the cotyledonary node and top symptoms no not appear until the stems are practically rotted off, then the plants wilt and dry rapidly (Fig. 1, D). No lesions were observed on the roots of Canada field pea. On Bard vetch the entire root system seemed to be rotted and partially destroyed, and the plants considerably weakened within two weeks (Fig. 1, B). On red clover the main tap root was rotted off and an increased number of lateral roots was produced on the tap root above the place where it had been severed (Fig. 1, E). On sour clover, very few single lesions were produced, but the main tap root and most of the fine feeder roots of infected plants were badly rotted. Berseem clover was apparently quite resistant as there were very few lesions on roots of affected plants.

There was no indication of host specialization in the strain of *Rhizoctonia solani* which causes alfalfa root canker. In inoculation tests it has been as pathogenic on other crop plants as on alfalfa. It is a very pathogenic strain capable of parasitizing several plant species.

SUMMARY

California Common variety of alfalfa was inoculated with isolates of *Rhizoctonia solani* from other plant species, and other plant species were inoculated with a strain of *R. solani* which causes a root canker of alfalfa.

Isolates from root cankers of alfalfa caused abundant root lesions when reinoculated to alfalfa. Isolates from other plant species produced no lesions on alfalfa roots, except isolate R–216 which was only weakly pathogenic and produced only a very few lesions on alfalfa.

Isolate 102, which causes root lesions on alfalfa, was pathogenic on roots of Bard vetch, Berseem clover, guar, Hubam sweetclover, sour clover, Madrid Evergreen sweetclover, and Cumberland red clover. It also was pathogenic on stems of Canada field pea.

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STUDIES ON ASCOCHYTA IMPERFECTA, A SEED- AND SOIL-BORNE PARASITE OF ALFALFA[1]

M. W. CORMACK[2]

(Accepted for publication June 2, 1945)

Ascochyta imperfecta Peck is best known as the cause of black stem of alfalfa. This disease, first described in New York in 1908 (16), is now widely distributed in many alfalfa-growing areas and is of considerable economic importance in certain regions (7, 13, 14, 17). Most of the damage occurs on varieties of *Medicago sativa*, but the pathogen can attack other species of *Medicago*, and also *Melilotus* and *Trifolium* (2). Non-leguminous hosts have not been reported.

Ascochyta imperfecta was officially identified as the cause of black stem of alfalfa throughout Canada in 1938 (3), when it was also found associated with a root rot of alfalfa and other legumes in Alberta. Subsequent studies have revealed that this fungus is commonly carried on the seed of alfalfa, as well as present in the soil of alfalfa fields, and that it can attack the seedlings under certain conditions. These previously unreported findings, and additional data obtained on the host range and life history of *A. imperfecta*, are presented in this paper.

OCCURRENCE OF ASCOCHYTA IMPERFECTA IN ALBERTA

Black Stem and Leaf Spot

Ascochyta imperfecta occurs commonly throughout Alberta on the stems and leaves of alfalfa. Infection is favored by cool, wet periods during the spring and fall. Although the damage is sometimes severe in certain seasons in isolated cases, it is not of major importance in Alberta. Actual killing of the plants, such as occurs in Kentucky (7), has not been observed. The most damage is caused by infection of the young shoots early in the season. Blackening of the stems occurs progressively and is usually not severe until late in the season in stands left for seed. Observational evidence indicates that severe infection of the flowering portion of the stem reduces the yield of seed. In Alberta, infection of the leaves is often more severe than that of the stems, and considerable defoliation may occur before the hay crop is cut.

Since the symptoms of black stem and leaf spot caused by *Ascochyta imperfecta* have been fully described by other workers (7, 13, 14, 17), only a few special features will be mentioned here. Stem lesioning ranges from slight spotting to complete discoloration (Fig. 1, A), and varies from light brown to deep black. It is often confined mainly to one side of the stem. When conditions are favorable, infection progresses up the stem to the racemes, even to the seed pods (Fig. 1, B, C). The pycnidia

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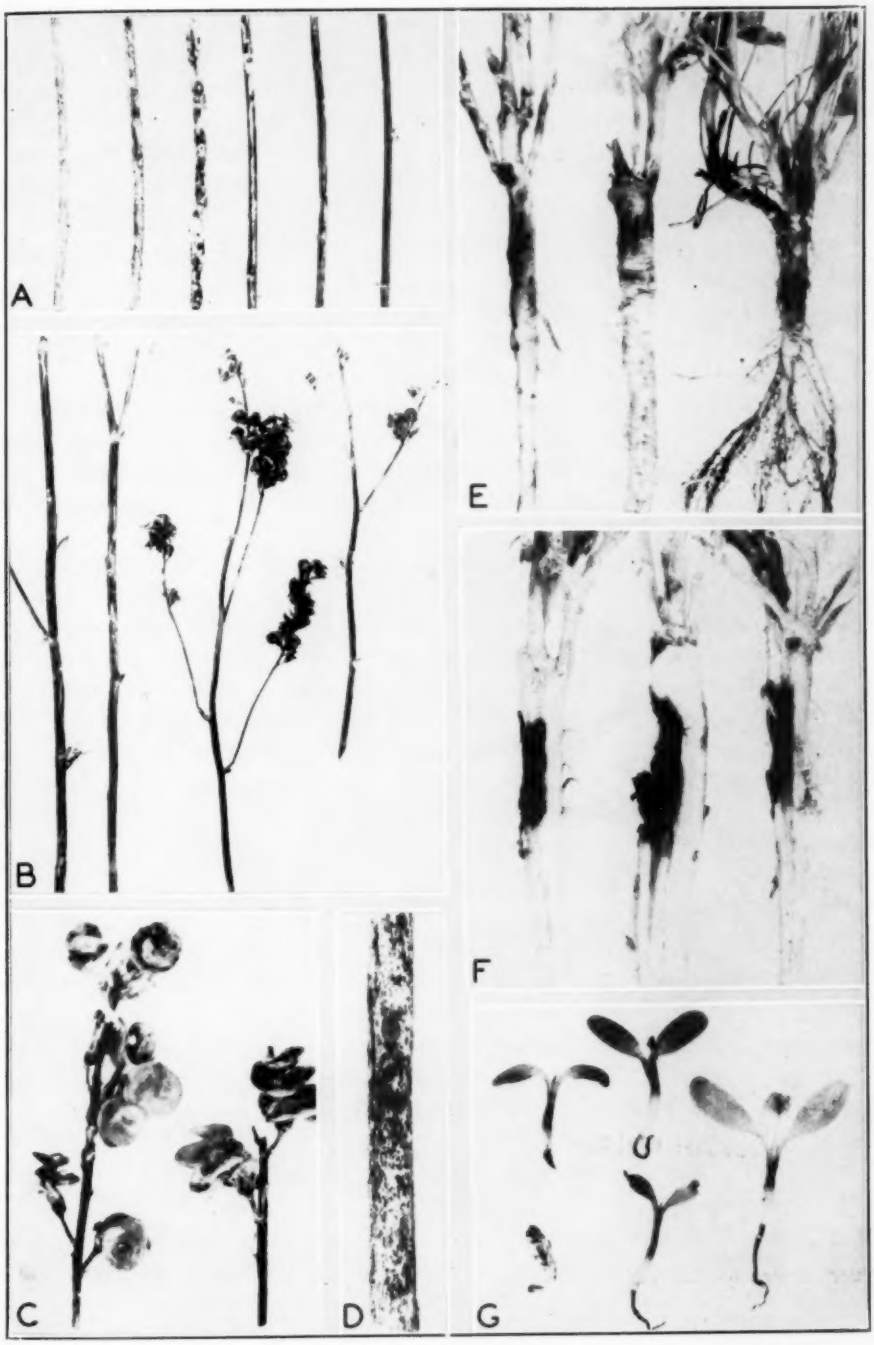

FIG. 1. Symptoms produced by *Ascochyta imperfecta*. A. Alfalfa stems infected to varying degrees. B. Entire alfalfa stem severely infected. C. Infected rachis and seed pods of alfalfa. D. Overwintered alfalfa stem with pycnidia. E. Affected roots of alfalfa, sweet clover, and red clover. F. Extent of rotting in roots of alfalfa, sweet clover, and red clover. G. Alfalfa seedlings from naturally infected seed.

of *A. imperfecta* are often absent or only sparingly borne on infected stems during the growing season. When present, they are most abundant in slightly dried, bleached areas, and are relatively scarce in the darker or blackened portions of the stem. They increase greatly in number after growth ceases in the fall and are usually very numerous on the dead, overwintered stems (Fig. 1, D). The only other disease of alfalfa which might be confused with black stem is bacterial stem blight caused by *Pseudomonas medicaginis* Sackett. The latter, however, occurs very rarely in Alberta and can be distinguished from black stem by the lighter colored lesions, usually covered with bacterial exudate.

The leaf symptoms produced by *Ascochyta imperfecta* are extremely variable but usually appear as irregular, dark-brown spots. The leaf spot caused by *Pseudopeziza medicaginis* (Lib.) Sacc. sometimes occurs on the same plants but can be distinguished by the smaller, circular lesions with raised central discs. *A. imperfecta* rarely produces mature pycnidia on the leaves. When infection is severe the spots coalesce and the leaves soon turn yellow and drop.

Ascochyta imperfecta was readily isolated from typical lesions on the stems and leaves of Siberian yellow-flowered alfalfa (*Medicago falcata*), as well as those of several varieties of *M. sativa*. A few isolates of *A. imperfecta* were also obtained from blackened stems of sweet clover (*Melilotus alba* and *M. officinalis*), but *Ascochyta lethalis* Ell. & Barth. was predominant on this host. Black-stem lesions on red clover (*Trifolium pratense*) and alsike clover (*T. hybridum*) yielded only unidentified species of *Ascochyta* and *Phoma*. Black-stem and leaf-spot symptoms were also fairly common on various noncultivated legumes. *A. imperfecta* was isolated occasionally from diseased stems of *Vicia americana* and from leaves of *Lathyrus spp.*, but unidentified species of *Ascochyta* predominated on these and other wild hosts.

Isolations from Roots

Ascochyta imperfecta was isolated from many diseased samples of alfalfa roots collected in various parts of Alberta, and from specimens sent from Saskatchewan. A few isolates were obtained from rotted roots of sweet clover. The fungus grew readily from diseased tissues picked out aseptically or surface-sterilized prior to plating. Usually it occurred in lesions with other root-rotting pathogens, especially *Cylindrocarpon Ehrenbergi* (4) and the low-temperature basidiomycete (5), and was detected only by isolation.

Symptoms of root attack typical of *Ascochyta imperfecta* were observed only in the relatively few cases where no other pathogen was present. The slightly sunken, dark-brown lesions usually occurred near the crown and rarely extended more than half way through the main root. Apparently infection occurred at the site of branch roots or wounds. The cracked or shredded tissues of these lesions sometimes contained a few pycnidia. Although the symptoms differ, this root rot is in some respects analogous

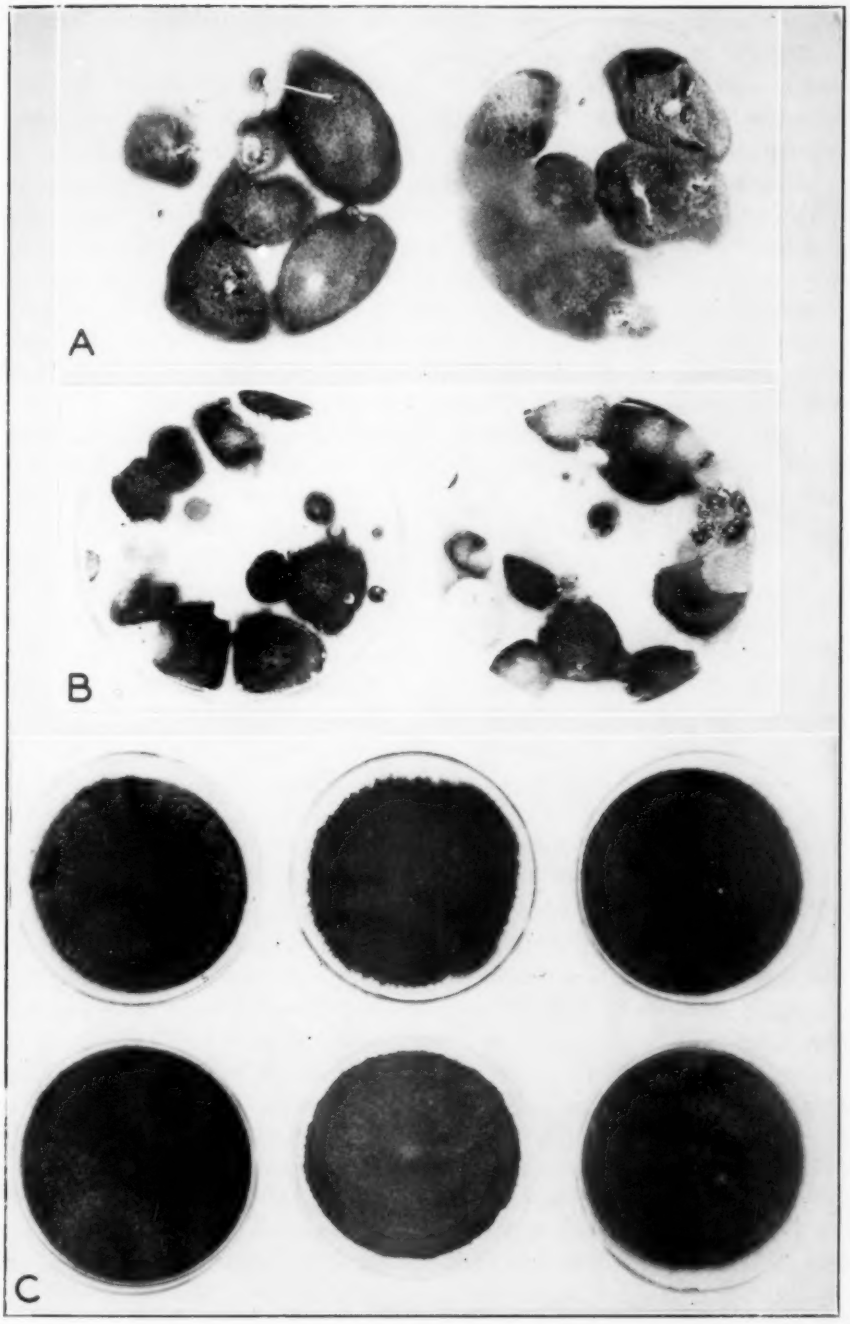

FIG. 2. A. *Ascochyta imperfecta* (dark-colored colonies) from naturally infected alfalfa seeds. B. Colonies in soil dilution plates from samples obtained from alfalfa fields. C. Ten-day-old cultures of 6 different isolates on potato-dextrose agar.

to the Stagonospora root rot of forage legumes described by Jones and Weimer (9).

Isolations from Seed

The fairly common occurrence of lesions on the racemes and seed pods of alfalfa suggested that the pathogen might be seed-borne. This was confirmed in seed isolation studies. The method used by Muskett and Malone (11) for the examination of flax seed proved most satisfactory. In most of the tests, 100 dry, untreated seeds of each sample were plated out on potato-dextrose agar. Surface disinfection was not practised, since it usually destroyed *Ascochyta imperfecta* and other fungi on the seed. After an incubation period of one week the characteristic, dark-colored colonies of *A. imperfecta* were easily recognized (Fig. 2, A), even when partly overgrown by *Alternaria spp.* or other saprophytic fungi. Seeds from all samples were also germinated on blotters or in soil, and isolations were made from the diseased seedlings.

TABLE 1.—*Prevalence of Ascochyta imperfecta and other pathogens in samples of alfalfa seed from different sources (1941 and 1942 crops)*

Source of seed	No. of samples examined	Number of samples infected[a]		
		Ascochyta imperfecta	*Stagonospora meliloti*	*Sclerotinia sclerotiorum*
Northern Alberta	35	18	3	3
Central Alberta	11	7	1	0
Southern Alberta	39	19	3	0
Other Provinces	12	5	0	1
Total number	97	49	7	3
Percentage	.....	50.5	7.2	3.1

[a] Containing one per cent or more of infected seed or sclerotia.

Isolations were made from seed harvested from 10 alfalfa stands having known amounts of black stem, and a direct relationship was found between seed infection and plant infection. The pathogen was isolated from 30 to 50 per cent of the seeds from fields in which infection was severe on the upper portions of the stems. When plant infection was slight, or was confined to the lower portion of the stems, from 90 to 100 per cent of the seeds escaped infection. It appears that seed infection occurs directly through the seed pods of severely diseased plants, and, to a lesser extent, by contamination during threshing and other operations.

The prevalence of *Ascochyta imperfecta* in alfalfa seed grown in 1941 and 1942 in various parts of Alberta was determined. The samples were obtained through the courtesy of the Plant Products Division, Production Service, Department of Agriculture, Calgary, Alberta, and were seed from foundation, elite, registered, and commercial stocks. Isolations were also made from a few samples received from the Provinces of British Columbia, Manitoba, Saskatchewan, and Ontario. *A. imperfecta* occurred in approximately one-half of the seed samples studied (Table 1). The pathogen was

isolated from seed obtained from all of the principal alfalfa-growing areas of Alberta, and also from at least one sample from each of the four other Provinces represented. These samples contained from 1 to 40 per cent of seeds infected with the fungus. Unfortunately, no information was available on the degree of infection in the stands from which they were harvested. The proportion of infected seed samples was approximately the same in the crop years of 1941 and 1942.

No association was found between the quality or appearance of the seed samples and their degree of contamination with *Ascochyta imperfecta*. Highly infected samples were found in all classes of seed examined. In tests with several samples containing 15 to 25 per cent of discolored, shrivelled, cracked, or green seeds, the pathogen was isolated as often from the sound, mature seeds as from the abnormal ones. It also occurred commonly on the hulls or other debris in poorly cleaned samples.

The results of dilution platings and microscopic examination of the sediment obtained by centrifuging samples of infected alfalfa seed strongly suggest that *Ascochyta imperfecta* persists mainly as mycelium on or in the outer portion of the seed coat. Spores were not observed in the sediment or washings from any sample. Moreover, pycnidia were not found on infected pods or seeds.

The only other pathogens found in alfalfa seed were *Stagonospora meliloti* (Lasch) Petrak (*Leptosphaeria pratensis* Sacc. & Briard), and *Sclerotinia sclerotiorum* (Lib.) de Bary. The sclerotia of the latter were mixed with the seed of a few samples. Neither of these pathogens was nearly as prevalent as *Ascochyta imperfecta* (Table 1). Also, no sample contained more than 2 per cent of seeds infected with *S. meliloti*. *Alternaria spp.* were most numerous among the various apparently saprophytic fungi isolated.

Ascochyta imperfecta was not found during the routine examination of several seed samples of sweet clover, red clover, and alsike clover. *Stagonospora meliloti*, previously reported as seed-borne by Jones (8), was found much more frequently in sweet-clover seed than in alfalfa seed.

Isolations from Soil

Samples of soil were taken in the fall from several alfalfa fields of varying age, cereal fields of known history, virgin prairie, and from virgin woods. They were taken one-half inch below the surface in all cases, and also at depths of 2 and 6 inches in the alfalfa fields. Soil dilutions were made, and plates of potato-dextrose agar poured in the usual manner. The colonies of *A. imperfecta* (Fig. 2, B) in each plate were counted after incubation for 7 days at room temperature.

Apparently *Ascochyta imperfecta* is much more prevalent in the soil of long-established stands of alfalfa than in the soil of those recently planted, and is most abundant at the one-half-inch depth (Table 2). It was isolated occasionally at the 2-inch depth, and, in the older stands,

TABLE 2.—*Occurrence and distribution of Ascochyta imperfecta in certain cultivated and uncultivated soils in Alberta*

Source of soil		Number of samples		Average number of colonies per plate		
Crop and history of field		Examined	Infested with *A. imperfecta*	0.5 inch depth	2 inch depth	6 inch depth
Alfalfa, 3 years old or older		10	10	9	0.5	0.1
Alfalfa, 1 year old		3	3	4	0.2	0
Cereals, 1st year after alfalfa		5	5	4	.....	.....
Cereals, 2nd year after alfalfa		2	0	.....	.....	.....
Cereals, 3rd year after alfalfa		2	0	.....	.....	.....
Wheat, various cereals		10	0	.....	.....	.....
Virgin prairie		3	0	.....	.....	.....
Virgin woods		3	0	.....	.....	.....

at the 6-inch depth. In the cereal fields, it occurred fairly commonly the first year after alfalfa sod was plowed, but was not obtained the second and third year. It is significant that the pathogen was not obtained from the soil of the cereal rotations or from the samples taken from the virgin prairie, and woods.

PATHOGENICITY OF ASCOCHYTA IMPERFECTA ON ALFALFA AND OTHER LEGUMES

Pathogenicity on Stems and Leaves

Representative isolates of *Ascochyta imperfecta* from the roots, stems, leaves, or seed of *Medicago*, *Melilotus* and *Lathyrus*, and from soil were tested for pathogenicity on the stems and leaves of alfalfa and other legumes. In greenhouse and field tests, vigorously-growing plants about three months old were sprayed or swabbed with a spore suspension of the pathogen (15). In the greenhouse, the inoculated and control plants were kept in a moist chamber for 3 days and were then placed under a bench until the symptoms developed. In the field, light frames covered with cheesecloth were placed over the plants for the entire period of incubation. A fairly high humidity was maintained within these cages by

TABLE 3.—*Relative virulence of isolates of Ascochyta imperfecta on the stems of Medicago, Melilotus, and Trifolium. (Greenhouse and field tests.)*

Isolates			Degree of stem infection[a]			
Source	Number tested	*Medicago sativa*	*Melilotus*		*Trifolium*	
			alba	*officinalis*	*pratense*	*hybridum*
Medicago, roots	2	S–M	T–S	T–S	S	T
Medicago, stems	4	S–M	S	T–S	S	T
Medicago, leaves	1	M	.....	.....	.....	.....
Medicago, seed	2	M	.....	.....	.....	.....
Melilotus, roots	1	S–M	.....	.....	.....	.....
Melilotus, stems	1	S–M	S	S	S	T
Lathyrus, leaves	1	M	.....	.....	.....	.....
Soil	1	M	.....	.....	.....	.....

[a] T—trace, S—slight, M—moderate.

constantly moistening the cheesecloth by means of cloth wicks leading into containers of water. After about 2 weeks the pathogen ceased to progress, and a final estimation was made of the degree of infection.

The typical symptoms of black stem and leaf spot were readily induced by the methods described, but infection was usually less severe than under natural conditions. Pycnidia seldom developed under conditions of artificial inoculation, but the pathogen was easily reisolated from the lesions on the stems and leaves.

On alfalfa stems, the isolates tested caused only slight to moderate infection (Table 3). The leaves, however, were slightly more susceptible. Four isolates, consisting of 2 from alfalfa stems, one from leaves of *Lathyrus* and one from the soil, caused moderate to severe defoliation of the plants.

A few of these isolates were also tested on other legumes (Table 3). A trace to slight infection developed on the stems and leaves of sweet clover, mainly in the vicinity of wounds. Some plants of *Melilotus alba* (variety Arctic) appeared to be slightly more susceptible than those of *M. officinalis* (variety Yellow Blossom). There was slight infection of the stems and leaves of red clover, and only a trace on alsike clover. Peas were not attacked by any of the isolates. These results are in general agreement with those reported by previous workers. Sprague (15) obtained slight infection of *Melilotus spp.*, *Trifolium pratense*, and *T. hybridum* with *Ascochyta imperfecta*. Toovey, Waterston, and Brooks (17) found the pathogen could attack *Medicago lupulina*, *Trifolium pratense*, and *Vicia sativa*, but not *T. repens*, *V. faba*, or *Pisum sativum*. *Medicago falcata* and *M. ruthenica* were added to the host range by Peterson and Melchers (13).

With regard to varietal resistance in alfalfa, studies started in the field are not yet complete. However, other workers (7, 13, 17) have reported differences in varietal reaction, and Koepper (10) has isolated a selection of Ladak that is highly resistant to *Uromyces striatus* and *A. imperfecta*.

Pathogenicity on Roots

The pathogenicity of *Ascochyta imperfecta* to roots of alfalfa and other legumes was studied in the field in summer and winter tests. Plants about one year old were inoculated by placing cotton pads containing oat-hull–soil inoculum against the partially bared tap roots (4). Since preliminary experiments showed that wounding greatly facilitated root infection by this fungus, the roots were slightly cut prior to inoculation. The roots of the noninoculated control plants were treated similarly. The plants inoculated during the growing season were dug up in about 4 weeks, but those inoculated in the fall were not examined until the following spring. The degree of infection of each root was estimated and expressed in percentage by means of the disease rating previously described (4).

Disease symptoms from the fall inoculations of the roots of alfalfa did not appear until the soil thawed out in the spring, and then the rotting

was slower than in comparable tests made during the summer. Apparently this fungus does not attack the roots at near-freezing soil temperatures, as is the case with certain low-temperature pathogens in Alberta (5). Only a slight to moderate amount of disease occurred at any time and the symptoms were similar to those previously described as occurring in nature (Fig. 1, E, F). No difficulty was experienced in reisolating the pathogen from the marginal tissues of the root lesions.

Certain isolates of the pathogen were more virulent than others in rotting the roots of alfalfa (Table 4), regardless of whether they were from the roots, stems, leaves, or seed of alfalfa, or from other hosts. That is to say, there was no evidence of specificity among the isolates in these respects.

Although the root-rot symptoms were similar on the various legumes (Fig. 1, E, F), *Medicago sativa* was usually more severely attacked than *Melilotus alba* (Table 4). The reaction of *Medicago falcata*, *Melilotus officinalis*, and *Trifolium pratense* to isolates from alfalfa roots was similar to that of *M. alba*. *T. hybridum* had a slightly lower disease rating.

Pathogenicity on Seedlings

Alfalfa seedlings were readily parasitized by *Ascochyta imperfecta* when naturally infected seeds were germinated on agar or blotting paper. The fungus grew out rapidly and either inhibited germination or attacked and rotted the young cotyledonary shoots. Healthy seedlings in contact with the diseased ones were also killed. Less seedling blight developed when the seeds were germinated under more natural conditions in sand or soil in the greenhouse. Fewer seedlings were attacked and infection was confined mainly to dark-brown lesions of varying size (Fig. 1, G). These lesions developed chiefly on the roots and hypocotyls, and to a lesser extent on the cotyledons. Severely infected seedlings usually did not emerge. Stunted and malformed seedlings were sometimes produced by weak or damaged seeds, but they lacked the distinct lesioning caused by the pathogen. When the lesioning was not extensive, the seedlings sometimes recovered and grew into fairly strong plants. Symptoms similar to those just described were produced by artificial inoculation of the seed or by infesting the soil with the pathogen, but the damage was usually more severe than that arising from naturally infected seed. In both cases the pathogen was consistently isolated from the lesioned seedlings.

The influence of soil temperature and moisture on infection of alfalfa seedlings by *Ascochyta imperfecta* was studied in soil-temperature-control tanks. Naturally infested seed was planted in crocks of steam-sterilized soil (3 parts of black loam and one part of sand) held at 4 soil temperatures, each averaging 11°, 17°, 21°, and 26° C. In one series at each temperature the soil was kept dry (approximately 40 per cent m.h.c.), and in the other series relatively moist (60 per cent m.h.c.). At each temperature and moisture, seed treated with Arasan, and untreated, was

TABLE 4.—*Relative virulence of isolates of Ascochyta imperfecta on the roots of Medicago, Melilotus, and Trifolium.* (*Field tests.*)

Isolates		General[a] pathogenicity tests						Special[b] test
Source	Number tested	*Medicago*		*Melilotus*		*Trifolium*		*Medicago*
		sativa	*falcata*	*alba*	*officinalis*	*pratense*	*hybridum*	*sativa*
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Medicago, roots	8	21	16	17	20	19	12	28.0
Medicago, stems	6	23	.....	14	.....	.....	.....	26.4
Medicago, leaves	2	19	.....	15	.....	.....	.....	18.6
Medicago, seed	2	22	.....	16	.....	.....	.....	29.4
Melilotus, roots	1	20	.....	17	.....	.....	.....	26.6
Melilotus, stems	1	20	.....	20	.....	.....	.....	.....
Lathyrus, leaves	1	24	.....	20	.....	.....	.....	30.8
Soil	2	21	.....	18	.....	.....	.....	33.8
Average rating	.....	21	.....	17	.....	.....	.....	27.7

[a] Average disease rating on 10 plants of one or more isolates from different sources.

[b] Average disease rating of 100 plants of Grimm alfalfa inoculated with one isolate from each source. F value for isolates 4.12, 5 per cent point 2.51, 1 per cent point 3.67. Difference required for significance (twice S.E. of difference) 6.4.

planted (20 seeds per crock), and in a third set the soil was infested at seed level with the pathogen (10 grams of soil inoculum per crock). Emergence was highest with the treated seed, lowest in infested soil, and intermediate when untreated seed was planted (Table 5). In the infested soil, the percentage of lesioned seedlings decreased with rise in temperature. Fewer seedlings emerged and more were diseased in the moist soil than in the dry soil at all temperatures except 26° C. In the steam-sterilized soil, lesioning occurred only on a few seedlings grown from untreated seed at 11° and 17° C. These results indicate that relatively cool, moist soil is most favorable for infection of alfalfa seedlings by *A. imperfecta*.

Ascochyta imperfecta was the only pathogen isolated from alfalfa seed

TABLE 5.—*Effect of soil temperature and soil moisture on pre- and post-emergence attack of alfalfa seedlings by Ascochyta imperfecta. (Naturally infected seed from the 1941 crop treated with Arasan, untreated, and planted in infested soil.)*

Temperature	Moisture	Emergence			Seedlings diseased		
		Seed		Soil infested	Seed		Soil infested
		Treated	Untreated		Treated	Untreated	
°C.		*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
11	Dry	68	56	47	0	2	7
	Moist	64	50	43	0	3	12
17	Dry	66	57	41	0	3	6
	Moist	60	54	36	0	3	8
21	Dry	63	52	52	0	0	4
	Moist	61	48	38	0	0	6
26	Dry	68	64	50	0	0	3
	Moist	70	63	50	0	0	3
Average		65.0	55.5	44.6	0	1.4	6.1

(Table 1) which proved parasitic to the seedlings. *Stagonospora meliloti* and *Sclerotinia sclerotiorum* occurred occasionally in the seed samples, but had no apparent effect on germination or growth. *Alternaria spp.* and other fungi and bacteria commonly isolated from the seed also appeared to be purely saprophytic.

SEED TREATMENT STUDIES

The effect of various fungicides on alfalfa seed infected with *Ascochyta imperfecta* was studied in laboratory, greenhouse, and field experiments. Treatments were made by thoroughly shaking the seed with a slight excess of the chemical dust, which was afterwards screened off. The relative efficiency of each treatment in destroying the pathogen was determined by plating out 100 seeds of each sample before and after treatment. In the greenhouse experiments, five 100-seed replicates of each sample and treatment were planted in sand in cardboard boxes. Similar experiments were conducted in the field in replicated 10-foot rows. Notes on emergence and disease development were taken 2 weeks after planting.

In the greenhouse experiments many samples of alfalfa seed were treated with Arasan, New Improved Ceresan (full strength and diluted to 1 per cent ethyl mercury phosphate with talc), Semesan, and Spergon. The results obtained in a representative test with 3 severely-infected samples are given in table 6. All fungicides tested were reasonably effective in inhibiting the pathogen, although it was usually isolated from a few seeds after treatment with Semesan and Spergon. Also, very few of the seedlings grown from treated seed were parasitized. Full strength New Improved Ceresan (5 per cent ethyl mercury phosphate), however, had an injurious effect on germination. Some samples were also slightly injured by Semesan and Spergon. With Arasan and diluted New Improved Ceresan (1 per cent ethyl mercury phosphate) there was no evidence of

TABLE 6.—*Effect of different chemical treatments on three samples of alfalfa seed naturally infected with Ascochyta imperfecta. (Greenhouse experiment.)*

Treatment	Seeds infected[a]			Emergence[b]			Seedlings diseased			Chemical injury
	Sample No.			Sample No.			Sample No.			
	1	2	3	1	2	3	1	2	3	
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	
Arasan	0	0	0	61.4	66.2	60.8	0	0	0	Trace
New Improved Ceresan, 1 Pct.	1	0	1	60.2	62.4	65.0	0	0	0	Trace
New Improved Ceresan, 5 Pct.	0	0	0	50.2	54.4	54.8	0	0	0	Moderate
Semesan	3	6	1	55.8	59.2	61.0	1	2	0	Slight
Spergon	4	1	2	61.0	60.2	59.8	1	0	1	Slight
Control (non-treated)	38	38	43	53.2	51.8	58.2	5	4	4	

[a] Based on isolations from 100 seeds of each sample.
[b] F value for treatments 12.11, 5 per cent point 2.35, 1 per cent point 3.29. Difference required for significance (twice S.E. of difference) 5.8.

chemical injury, and emergence in all three samples, when treated, was significantly higher than in the nontreated controls. As previously noted (Table 5), Arasan also increased the emergence and prevented blight of the seedlings under varying conditions of soil temperature and soil moisture.

In field experiments with a large number of samples the effects of seed treatment were much more variable than in the greenhouse. Treatment with Arasan, New Improved Ceresan, Semesan, and Spergon increased emergence in some samples but not in others. The beneficial effect was not entirely due to control of the pre-emergence blight caused by *Ascochyta imperfecta*, since seed treatment stimulated germination in samples containing both high and low percentages of infected seed. The pathogen apparently caused at least part of the pre-emergence blight that occurred in some of the nontreated controls, but it did not produce lesions on the emerged seedlings in the field. Full strength New Improved Ceresan had only a slight detrimental effect on germination in the field, and the other treatments apparently caused no injury.

LONGEVITY OF THE PATHOGEN

The longevity of *Ascochyta imperfecta* in dry stems and leaves of alfalfa was studied by isolating at yearly intervals from herbarium specimens stored at room temperature. Spore and tissue isolations were made from each sample. The viability of the spores from the 10 samples of stems studied remained high for about 4 years after date of collection, after which it decreased rapidly. In 2 samples a few spores were viable after 6 years, but none germinated at the end of 7 years. Isolations from tissues were made with great difficulty from stems stored 5 years, and none were obtained by the end of the sixth year. The pathogen also remained viable for about 5 years in the tissues of infected alfalfa leaves.

In the case of alfalfa seed, isolations were made at yearly intervals from samples naturally infected with *Ascochyta imperfecta*. These samples were stored in seed envelopes at room temperature. The 16 samples harvested in 1941 showed an average infection of the seeds of 25 per cent after being stored for one year, 16 per cent after 2 years, and 6 per cent after 3 years. However, at the end of 3 years the fungus was not isolated from samples which originally contained less than 5 per cent of infected seeds. The most severely infected sample contained about 54 per cent of infected seeds during the first year of storage, 40 per cent after 2 years, and 20 per cent after 3 years. The pathogen remained viable for 3 years in 2 out of 4 samples from the 1940 crop, and for 4 years in only one of the samples. In the latter sample the original infected seed content of about 25 per cent decreased to 2 per cent during the 4-year period. In comparison with these results, Crosier (6) found that the number of seeds of hairy vetch infected with *Ascochyta pisi* decreased continuously during a storage period of 5 years. Sprague (15), however, isolated this pathogen from 9-year-old seed of *Vicia faba*. Longevity tests on *A. imperfecta* have apparently not been previously reported, and the present studies are being continued.

TAXONOMY

Considerable confusion exists in the literature concerning the identity of the causal agent of black stem of alfalfa. It was first described as *Ascochyta imperfecta* by Peck (12) in 1911, and this name was recognized by Sprague (15) in 1929 in his study of the leguminous Ascochytae. Johnson and Valleau (7), in 1933, however, attributed a similar disease of alfalfa in Kentucky to *Phoma medicaginis* Malbr. & Roum., and the latter name was accepted by Remsberg and Hungerford (14) and other workers. In 1936, Toovey, Waterston, and Brooks (17), in Britain, made a careful study of all available specimens and cultures, including the pathogen from Kentucky, and concluded that they all belonged to the same species and should be properly referred to as *Ascochyta imperfecta* Peck. These workers suggested that certain other species of *Ascochyta*, *Diplodia*, *Phoma*, and *Phyllosticta* described on alfalfa in Europe might

also be identical with *A. imperfecta*. This name has been used in the Canadian plant disease survey reports since 1938 (3), and has now become generally recognized in the literature.

Description of the Alberta Isolates

Cultures of *Ascochyta imperfecta* isolated from various hosts and sources in Alberta conformed fairly closely to the original species description by Peck (12), and also fell within the range of the characteristics described by Toovey, Waterston, and Brooks (17). These isolates, however, differed markedly from each other in certain cultural characteristics (Fig. 2, C). The colonies ranged from light grey to dark-brownish grey, usually with a characteristic olive tinge. Some isolates grew much more rapidly than others, possibly due to different rates of staling. The colony diameter of the different isolates ranged from 25 to 65 mm. after incubation for 7 days at 22° C. Pycnidia varied in number, size, color, and in position in the culture. They were usually abundant, but certain isolates characteristically produced them very sparingly, even when cultured on sweet-clover stems or other media particularly favorable for their fruiting. The pycnospores were continuous or one-septate, depending on the isolate and on cultural conditions, and were generally larger than those produced on the host. Chlamydospores were very numerous, and a few large, brownish, one-septate, spore-like bodies sometimes occurred at the surface of the medium. The white crystal aggregates described by other workers (7, 17) were produced in the medium in varying numbers by different isolates.

Variants frequently occurred in the form of sectors or patches in test-tube and plate cultures (Fig. 2, C). These variants differed from the parent culture in color, growth rate, and pycnidial production. It is possible that variants of this fungus also occur commonly in nature, which would account for many of the differences observed between isolates.

There was no evidence of any relationship between the cultural characteristics of an isolate and its source. For example, the full range of characteristics described occurred among the isolates from both stems and roots of alfalfa. The composition of the medium as well as various environmental conditions had a marked influence on the cultural behavior of the fungus, but, in general, they did not mask the differences between isolates.

In temperature studies, most of the Alberta isolates of *Ascochyta imperfecta* grew best at 20°–22° C., which is in close agreement with the results of Peterson and Melchers (13). Pycnidia and spores generally developed most rapidly at about 17° C. At 5° C. growth was relatively slow and sparse, but the pycnidia were eventually as numerous as at higher temperatures. When cultured on sweet-clover stems, most isolates produced numerous pycnidia and caused a blackening of the stems that was particularly evident at 17° C.

The Perfect Stage

Over 50 samples of overwintered stems of alfalfa collected in different sections of Alberta were examined in an effort to find the perfect stage of *Ascochyta imperfecta*. Also, several unsuccessful attempts were made to induce perithecial development by subjecting infected stems and cultures to varying conditions of temperature, moisture, and light.

Perithecia of *Leptosphaeria, Mycosphaerella, Pleospora,* and other fungi were not infrequently found in association with the pycnidia of *Ascochyta imperfecta* on the overwintered stems. No attempt was made to identify most of these fungi, since cultural tests indicated that they had no genetic connection with the pathogen. However, a special study was made of a species of *Pleospora* found on 3 of the overwintered samples. This fungus corresponded very closely to the description of *P. rehmiana* (Staritz) Sacc., reported by Remsberg and Hungerford (14) as the perfect stage of the black-stem pathogen (*Phoma medicaginis* Malbr. and Roum.) in Idaho. As mentioned above, this fungus is now considered identical with *A. imperfecta*. These workers found perithecia of *P. rehmiana* on old stems of alfalfa, and in sweet-clover-stem cultures of the pathogen held for 3 months at 0°–5° C. They stated that the original single-ascospore isolates were somewhat different from single-spore isolates of the pathogen, but that typical pycnidia and pycnospores developed after culture on sweet-clover stems. Single-ascospore isolates from perithecia on Alberta specimens, however, developed into cultures unlike those of *A. imperfecta*. These isolates also retained their identity and failed to produce pycnidia or pycnospores under a wide range of conditions, which included culture for several months on sweet-clover stems at 0°–25° C. Perithecia, asci, and ascospores, closely resembling those found in nature, eventually developed in sweet-clover-stem cultures held at 0° to 18° C.

The Alberta isolates of the *Pleospora rehmiana*-like fungus also failed to attack the stems and leaves of alfalfa in several inoculation experiments. They caused very slight infection of alfalfa and sweet-clover roots, and the original fungus was reisolated from the lesions.

From this evidence it is concluded that the *Pleospora sp.* resembling *P. rehmiana,* occasionally found on overwintered stems of alfalfa in Alberta, has no connection with *Ascochyta imperfecta*. Johnson and Valleau (7) were also unable to establish a genetic connection between *Pleospora* and the black-stem pathogen in Kentucky.

DISCUSSION

Present evidence indicates that *Ascochyta imperfecta* is primarily a parasite of the stems and leaves of alfalfa, and is of doubtful importance on other naturally infected hosts. Although able to cause root rot of legumes under experimental conditions, it usually occurs in nature in root lesions produced by other fungi. For this reason it is regarded as of less importance in Alberta than any of the root-rotting pathogens pre-

viously reported (5). However, there are indications that it may increase the root damage started in the early spring by the low-temperature fungi with which it is usually associated. Infection of alfalfa seedlings by *A. imperfecta* is also of relatively minor importance under average field conditions in Alberta. Pre-emergence attack, however, sometimes results in lowered seed vitality, and this aspect requires further study.

Diseased alfalfa stems, on which numerous pycnidia of *Ascochyta imperfecta* develop in the late fall, are undoubtedly the most important source of inoculum. Under moist conditions in the spring the spores exude from these pycnidia and are readily disseminated by rain drops to start a new cycle of infection on the young growth. The frequent occurrence of *A. imperfecta* in lesions at or near the crowns suggests that infected crop residue decaying near the surface is also the source of inoculum for alfalfa roots. It is natural that as alfalfa stands become older and plant debris accumulates, the pathogen increases in abundance in the surface soil and may also spread downward to a limited extent. Apparently it is unable to persist very long in the soil after alfalfa has been plowed, which indicates the value of crop rotation. However, it may remain viable for at least 5 years on dried stems and leaves of alfalfa, and for about 3 years on the seed. This suggests that the hay, meal, and seed obtained from infected alfalfa stands may harbor the fungus and aid in its dissemination. Infected seed, in particular, may serve to introduce the pathogen into areas or fields where it is not already present. Hence, it is possible that the distribution of infected seed may be partly responsible for the general prevalence of stem and leaf infection by *A. imperfecta* in alfalfa stands of all ages.

As suggested by Allison and Torrie (1), the chemical treatment of alfalfa seed requires much further study before it can be recommended as a general practice. The effect of fungicides on seed germination and on the nodule-producing bacteria are factors that must be considered. In Alberta, seedling blight of alfalfa caused by *Ascochyta imperfecta* or other seed- and soil-borne pathogens does not yet seem of sufficient importance to warrant general seed treatment. Further investigation is required, however, to determine the possible value of testing alfalfa seed for the presence of *A. imperfecta*, and of treating heavily-infected samples in order to reduce the spread of the pathogen.

SUMMARY

In field, greenhouse, and laboratory studies, *Ascochyta imperfecta*, the fungus causing black stem of alfalfa, parasitized the roots of alfalfa and other legumes, and also seedlings of alfalfa, and it was both seed- and soil-borne.

Medicago sativa was more susceptible to stem, leaf, and root infection than any other host studied. *Melilotus spp.* and *Trifolium pratense* were slightly susceptible, and *T. hybridum* rather resistant. Occasionally the

pathogen was isolated from stems of *Vicia americana* and leaves of *Lathyrus spp.*

Cool, moist conditions were most favorable for disease development on stems, leaves, and seedlings of alfalfa. The pathogen did not attack the roots of dormant plants at soil temperatures near freezing.

The degrees of seed and stem infection were directly correlated. *Ascochyta imperfecta* was isolated from as much as 40 per cent of the seeds of 49 out of 97 random samples of alfalfa seed. New Improved Ceresan, diluted to one per cent ethyl mercury phosphate, and Arasan were more effective than other seed treatments tested.

The fungus was prevalent in the surface soil of alfalfa fields, but disappeared 2 years after the sod was plowed. Moreover, it was not isolated from the soil of cereal rotations, virgin prairie, or virgin woods. It persisted on dry stems and leaves of alfalfa for at least 5 years, and on alfalfa seed for about 3 years.

There was no evidence of host specificity, since isolates of *Ascochyta imperfecta*, obtained from various hosts, different parts of the same host, and from soil, exhibited similar differences in virulence and cultural characteristics.

Various Ascomycetes, including a *Pleospora* closely resembling *P. rehmiana*, were found associated with the pycnidia of *A. imperfecta* on overwintered stems of alfalfa, but, in no case was proof of a genetic connection established.

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AMERICAN PHYTOPATHOLOGICAL SOCIETY

1945 Meeting Canceled

The Office of Defense Transportation has announced that travel restrictions will not be lifted for several months after official declaration of the end of the war. For this reason the Council voted that it was inadvisable to hold the 1945 meeting, scheduled for December 10–12, in Cincinnati, Ohio.